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Abstract: Ornithine transcarbamylase (OTC) deficiency is an X-linked trait that accounts for nearly half of all inherited disorders of the urea cycle. OTC is one of the enzymes common to both the urea cycle and the bacterial arginine biosynthesis pathway, however the role of OTC has changed over evolution. For animals with a urea cycle, defects in OTC can trigger hyperammonemic episodes that can lead to brain damage and death. This is the fifth mutation update for human OTC with previous updates reported in 1993, 1995, 2002, and 2006. In the 2006 update, 341 mutations were reported. This current update contains 417 disease causing mutations, and also is the first report of this series to incorporate information about natural variation of the OTC gene in the general population through examination of publically available genomic data, and examination of phenotype/genotype correlations from patients participating in the Urea Cycle Disorders Consortium Longitudinal Study and the first to evaluate the suitability of systematic computational approaches to predict severity of disease associated with different types of OTC mutations.

Editorial Board
Journal of Genetics and Genomics

April 4, 2015

Dear Editorial Board:
Attached please find our substantially revised article to address the comments from the Editor entitled **Genotype Phenotype Correlations in Ornithine Transcarbamylase Deficiency: A Mutation Update** in which we describe the spectrum of mutations associated with disease due to mutations in ornithine transcarbamylase.

This is the first systematic application of computational approaches to predict severity of disease associated with mutations in ornithine transcarbamylase. We are submitting this article as part of the 2014 International Conference on Arginines and Pyrimidines held in Oxford University hosted by Professor Ji-Long Liu, and believe that this work has merit with respect to medical genetics, and bioinformatics appropriate to your Journal.

Thank you for the extensive comments and suggestions.
We have addressed all the areas where it was suggested to polish the writing, and made all the other editorial changes.

On page 7, there were comments asking for references for OTC mutations. These data are reported for the first time in this article based on our analysis of the UCDC cohort

We have moved all suggested tables to Supplementary Material
We have moved and renamed Methods to Supplementary Data, and put it at the end of the manuscript.

A comment was made regarding the text in the Acknowledgement.
As a member of the Urea Cycle Disorders Consortium, we are required to use this text to describe the Consortium and the funding sources that helped collect and manage the patient samples for this study.

Sincerely,
Hiroki Morizono, PhD
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***Detailed Response to Reviewers-is same as Cover Letter**

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***Conflict of Interest**

Click here to download Conflict of Interest: CONFLICT OF INTEREST STATEMENT.docx

We thank the reviewer for their helpful suggestions and critiques. We have addressed all of the points raised as indicated below:

Page 11: "We have previously characterized several OTC mutations based on the enzyme kinetics of
recombinant proteins [REF]."
Valid reference(s) should be added.

Thank you for catching the missing references.

Page 11: "The PALO affinity column approach is not effective for mutations in the active site, and affinity tags are not able to discriminate between soluble trimers and small protein aggregates."
Not obvious what a PALO affinity column is?

We have rewritten the section describing the PALO (N-phosphonoacetyl-L-ornithine) affinity column.

I am also wondering if nobody else than the authors themself attempted biochemical methods for diagnosis? This is the impression I get from these two sentences that were added upon my previous comments. I would strongly recommend the authors to seriously rewrite and expand this part including references.

Although there are numerous biochemical diagnostic reports in the literature, with many from our clinical lab, most are based on crude liver homogenated prepared from biopsy tissue. There are some reports from transplant and cadaver livers where the OTC enzyme was purified. We do appear still to be the only group where wild-type and mutant human OTC was overexpressed, and purified to homogeneity. We hope we have sufficiently rewritten this section to address that concern-we had been thinking about purified recombinant protein for detailed biochemical and biophysical analysis, and these experiments were not for diagnosis, but for characterization, and scaling expression levels for protein crystallization.

Figure 1. Schematic of a hepatocyte highlighting the various enzymes, transporters, and metabolites of the urea cycle. Shown in yellow is a mitochondrion containing theproximal enzymes NAGS, CPSI, OTC, and the ORNT and Citrin transporters. Citrulline is transported out of the mitochondrion, and converted by ASS, ASL, ARG1 to produce urea and regenerate ornithine that is reutilized by OTC.
Please state the full names of the enzymes (e.g. ASS etc) and compounds (e.g. NAG) that are not introduced in the main text - both from figure legend as well as figure itself.

For Figure 1, we tried to comply with using the full names of the enzymes in the figure, but this resulted in an extremely crowded image that was difficult to

decipher. We have placed a key in the Figure and have used the full names in the Figure Legend, and provided the abbreviations there.

Genotype–Phenotype Correlations in Ornithine Transcarbamylase Deficiency: A Mutation Update

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Key words: Ornithine transcarbamylase; Mutation; Ornithine transcarbamylase deficiency; Urea cycle; Hyperammonemia

Abstract

Ornithine transcarbamylase (OTC) deficiency is an X-linked trait that accounts for nearly half of all inherited disorders of the urea cycle. OTC is one of the enzymes common to both the urea cycle and the bacterial arginine biosynthesis pathway; however, the role of OTC has changed over evolution. For animals with a urea cycle, defects in OTC can trigger hyperammonemic episodes that can lead to brain damage and death. This is the fifth mutation update for human OTC with previous updates reported in 1993, 1995, 2002, and 2006. In the 2006 update, 341 mutations were reported. This current update contains 417 disease-causing mutations, and also is the first report of this series to incorporate information about natural variation of the *OTC* gene in the general population through examination of publically available genomic data and examination of phenotype/genotype correlations from patients participating in the Urea Cycle Disorders Consortium Longitudinal Study and the first to evaluate the suitability of systematic computational approaches to predict severity of disease associated with different types of OTC mutations.

Key Words: ornithine transcarbamylase, mutation, urea cycle

INTRODUCTION

Ornithine transcarbamylase (OTC, MIM# 311250, RefSeq NP_000522) is a mitochondrial urea cycle enzyme that catalyzes the reaction between carbamyl phosphate and ornithine to form citrulline and phosphate. OTC is essential for the conversion of neurotoxic ammonia into non-toxic urea in mammals and other ureotelic animals (Fig. 1, (Brusilow and Horwich, 2001). In microbes and plants, OTC catalyzes the sixth step of arginine biosynthesis (Glansdorff and Xu, 2006) while the function of OTC in animals that excrete nitrogenous waste as either uric acid or ammonia remains to be elucidated.

The human *OTC* gene is located on the X-chromosome within band Xp21.1 (Lindgren et al., 1984). Ten exons and nine introns spanning 73 kb comprise the human *OTC* gene with an open reading frame of 1062 nucleotides (Horwich et al., 1984; Hata et al., 1986). The precursor protein contains 354 amino acids and has a calculated molecular weight of 39.9 kDa. Upon import into the mitochondria, a 32 amino acid N-terminal leader sequence is removed in two steps (Horwich et al., 1986). The mature OTC protein contains 322 amino acids and has a calculated molecular weight of 36.1 kDa. The functional OTC is a homotrimer; it has three-fold symmetry and three active sites, located at the interface between the protein monomers (Shi et al., 1998). OTC is expressed in the liver, the only organ that expresses all urea cycle enzymes, and in the intestinal mucosa. In the liver, ammonia is converted into urea, while in the intestinal mucosa, where N-acetylglutamate synthase, carbamylphosphate synthetase and OTC are also found, conversion stops at citrulline, a precursor of arginine and an intermediate in NO-signaling (Brusilow and Horwich, 2001).

OTC deficiency (OTCD) is the most common inherited defect of ureagenesis because the *OTC* gene is located on the X-chromosome. OTCD accounts for about half of all urea cycle

defects (Brusilow and Horwich, 2001; Seminara et al., 2010). The estimated prevalence of OTCD is one in 14,000 (Brusilow and Maestri, 1996), but more recent estimates based on a review of national medical records and comparisons of newborn screening data with the number of patients with urea cycle disorders indicate a prevalence of one in 62,000–77,000 (Dionisi-Vici et al., 2002; Keskinen et al., 2008; Balasubramaniam et al., 2010; Summar et al., 2013). Most of the patients with OTCD are hemizygous males; approximately 20% of female carriers of *OTC* mutations also present symptoms of OTCD (Maestri et al., 1996; Maestri et al., 1998). The onset of OTCD symptoms is extremely variable. Heterozygous females and males with partial defects in the *OTC* can present later in life and well into adulthood, while hemizygous males with complete OTCD present with acute hyperammonemia within the first week of life (Hudak et al., 1985; McCullough et al., 2000). Neonatal presentation usually correlates with the absence of liver OTC activity (Tuchman et al., 1998) and null alleles (McCullough et al., 2000). Approximately 50% of patients with partial OTCD present later in life or even in adulthood (Finkelstein et al., 1990; Tuchman and Holzknecht, 1991). The clinical symptoms of OTCD result from the toxic effects of ammonia on the brain and can include recurrent vomiting, a clinical picture resembling Reye syndrome (Glasgow and Middleton, 2001), neurobehavioral changes or seizures. In addition to elevated plasma ammonia, biochemical symptoms of OTCD include elevated plasma glutamine, low or absent plasma citrulline and elevated urinary orotic acid, which distinguishes OTCD from other proximal urea cycle disorders.

This is the fifth mutation update for the human *OTC* gene (Tuchman and Plante, 1995; Tuchman et al., 1998; Tuchman et al., 2002; Yamaguchi et al., 2006). In addition to 417 disease-causing mutations, this report contains information about natural variation in the human *OTC* gene and the severity of disease associated with different types of *OTC* mutations.

MUTATIONS AND POLYMORPHISMS IN THE *OTC* GENE

A total of 417 disease-causing mutations in the *OTC* gene, including 29 mutations reported here for the first time, are listed in Table S1. Twenty-three of the newly reported mutations were identified by the longitudinal study of urea cycle disorders (Batshaw et al., 2014). Previously undetected chromosomal defects in intronic and regulatory regions of the *OTC* gene can now be diagnosed due to advances in sequencing technologies. Fifty-two patients with OTCD due to deletions, duplications or complex rearrangements involving *OTC* gene have also been identified (Table S2). Of the identified disease-causing mutations reported for *OTC*, the majority are amino acid replacements, followed by RNA splicing defects, premature protein terminations and deletions (Table 1). Many sequence changes within the *OTC* coding sequence result in more than one missense mutations of the same codon and some, but not all, of these mutations occur within codons that overlap with CpG dinucleotides on both strands (Fig. 2).

A complete loss of OTC function due to large deletions, frameshift and nonsense mutations as well as missense mutations that abolish either enzymatic activity or folding of OTC is known to cause neonatal onset disease in hemizygous males and symptoms of OTCD in some heterozygous females (Tuchman, 1993; Tuchman et al., 1997; Tuchman et al., 2002; Yamaguchi et al., 2006). Late onset disease in hemizygous males is caused by missense mutations that retain some OTC activity but have destabilized protein or lower enzymatic activity or decreased substrate affinity. A few female carriers of hypomorphic *OTC* alleles can also present with symptoms of OTCD (Pinner et al., 2010). Disease presentation and severity in OTCD patients with missense mutations were rationalized by assessing the position and function of the affected amino acid in the OTC enzyme (Tuchman et al., 1997; Tuchman et al., 2002). However, these studies did not attempt to find predictors of the severity of OTCD based on the biochemical

properties of mutant OTC. As patients undergoing whole exome sequencing become more common, uncovering OTC variants is only likely to increase, making it important to have an understanding of the relationship between specific mutations and disease. Buried amino acids have more stringent steric restrictions in a folded protein, and examination of the degree of amino acid conservation, and solvent accessibility of mutation sites identified in male and symptomatic OTCD patients revealed that the largest numbers of missense mutations affect the most conserved and least solvent exposed residues (Fig. 3A and C). In both symptomatic females and male patients (Fig. 3B), the second most common missense mutations affect moderately conserved residues that also have little solvent accessible surface (Fig. 3E–G). These moderately conserved residues tend to be buried in the protein interior (Fig. 3F and G). Most mutant OTC proteins found in patients with OTCD are predicted to be less stable that the wild type (Fig. 4A and B) but the distributions of calculated differences in free energies between mutant and wild type OTC differed between the two methods for calculation used here (Fig. 4C and D). Most symptomatic females had missense mutations that were highly destabilizing to the mutant protein while there was a paucity of such mutations in males with late onset OTCD (Fig. 4D).

In addition to disease-causing mutations, 44 rare sequence variants (MAF < 1%) have been found in the *OTC* gene (Table S3). In previous reports, 12 of these SNPs were considered to be polymorphisms (non-disease causing) because they have been found through screens of healthy individuals ((Hata et al., 1988; Matsuura et al., 1993; Tuchman and Plante, 1995; Plante and Tuchman, 1998; Climent and Rubio, 2002a, b; Tanaka et al., 2005). The remaining 32 rare SNPs were found through querying the 1000 Genomes project and NHLBI Exome Variant Server (ESP6500SI-V2); 18 rare SNPs have been found in heterozygous females and are predicted to affect either splicing of the *OTC* mRNA or function of the OTC enzyme (Kumar et

al., 2009; Adzhubei et al., 2010; Schwarz et al., 2010) suggesting that female carriers of these alleles might be predisposed to OTCD. Eighteen common polymorphisms (MAF ≥ 1%) have been found in the regulatory region, within exons and in the introns in close proximity of intron/exon boundaries (Table 2). Allele frequencies of the majority of common polymorphisms that were found through population screens by individual researchers (Tuchman and Plante, 1995; Plante and Tuchman, 1998; Azevedo et al., 2002a; Azevedo et al., 2002b; Climent and Rubio, 2002a; Azevedo et al., 2003) are similar to those reported in the dbSNP, 1000 Genome project and NHLBI Exome Variant Server. Two variants, rs73196229 (c.299-8A>T) and rs1800326 (c.387-7G>A), however, appear to differ in populations of healthy individuals (1000 Genomes projects, ESP6500SI-V2 and Azevedo et al(Azevedo et al., 2002a). and among patients suspected of OTCD (Tuchman and Plante, 1995; Plante and Tuchman, 1998; Climent and Rubio, 2002a). It is possible that these two variants may affect splicing of the *OTC* mRNA and result in symptoms of OTCD.

CLINICAL, DIAGNOSTIC, AND BIOLOGICAL RELEVANCE

Mutations that completely abolish either *OTC* mRNA production and processing or OTC enzymatic activity manifest with acute neonatal onset hyperammonemia. Individuals with sequence variants that allow residual OTC activity can present with hyperammonemia at any point in life, while others remain asymptomatic. In patients with late onset OTCD, acute hyperammonemia can be triggered by a high protein meals (Ben-Ari et al., 2010; Thurlow et al., 2010; Cavicchi et al., 2014), fasting (Marcus et al., 2008), infections (McGuire et al., 2013), invasive medical procedures (Chiong et al., 2007; Hu et al., 2007; Bezinover et al., 2010), chemotherapy (Lipskind et al., 2011; Cavicchi et al., 2014), or other environmental insults that result in increased protein catabolism and ammonia production (Seminara et al., 2010). Missense

mutations that cause partial OTCD reduce OTC enzymatic activity or stability while mutations in the vicinity of consensus splice sites can potentially affect mRNA processing and result in decreased abundance of the OTC enzyme. Large phenotypic heterogeneity is observed among patients with hypomorphic *OTC* alleles, even among members of the same family. This is likely due to environmental factors and genetic modifiers, which are poorly understood at present. A better understanding of the correlation between genotype and phenotype of OTCD is needed to interpret the effects of *OTC* sequence variants discovered through sequencing of whole exomes and genomes and could be achieved by studying natural history of OTCD (Batshaw et al., 2014).

GENOTYPE AND PHENOTYPE OF PATIENTS IN THE LONGITUDINAL STUDY OF OTCD

We wanted to determine whether the severity of OTCD, measured by disease onset, biochemical markers and intellectual outcomes, could be correlated with changes in the OTC protein that can be calculated and/or predicted from the known three dimensional structure of the OTC trimer (Ding and Dokholyan, 2006; Yin et al., 2007a, b; Adzhubei et al., 2010). A total of 398 participants with OTCD and their asymptomatic relatives have been enrolled in the longitudinal study of urea cycle disorders for the study of natural history of OTCD. Gender, disease onset and liver transplant status have been recorded for each participant. The *OTC* genotypes were determined for 293 study participants (197 females, 80 males and 16 deceased patients without gender information). Table S4 lists the participants' gender, onset of their disease and whether they received a liver transplant. In addition to the study participants listed in Table S4, six asymptomatic males and 88 asymptomatic females as well as six males and 12 female study subjects with unknown disease onset have been enrolled in the longitudinal study of OTCD. Participants in the study are examined regularly; biomarkers of liver function are measured at

each visit (Batshaw et al., 2014); neuropsychological evaluations of study participants are performed when they are six months, four, eight, 15 and 18 years old and once in adulthood (Weisbren et al., 2014). Of the 293 participants with a known genotype, 18 participants have large deletions of the whole or parts of *OTC* gene while remaining 275 have either point mutations or small insertions and deletions. The majority of participants, 66 male and 140 female, have missense mutations, 26 participants (3 male and 23 female) have nonsense mutations, 11 participants (2 male and 9 female) have frameshift mutations and 14 (5 male and 9 female) have mutations that affect splicing. Three female participants have mutations within their stop codon that result in extending of the OTC protein and one male patient has a 9 bp in frame insertion that results in the insertion of three amino acids in the OTC protein. Many point mutations occur repeatedly and they are in codons that either contain or overlap with CpG dinucleotides (Fig. 5).

Several measures of mutation severity were used to assess whether certain types of mutations are associated with neonatal or late onset OTCD. A majority of both male and female study subjects had OTCD due to missense mutations (Table 3). The second largest group of study participants had defects that resulted in the complete loss of functional OTC enzyme. One male study subject with late onset OTCD had a mutation in a consensus splice site that would have been expected to result in a neonatal presentation, which could not be determined whether the late onset was due to partial splicing of the RNA or due to somatic mosaicism (Table 3). The severity of missense mutations was assessed using conservation and solvent accessible area of the replaced amino acid, calculated destabilization of mutant proteins and their SIFT and PolyPhen2 scores (Figs. S1–S3). Most missense mutations found in males with neonatal OTCD and females with late onset OTCD were found to be replacements of highly conserved amino acids, while males with late onset disease and asymptomatic males and females had replacements

of less conserved residues (Fig. S1A and B). Most mutated residues were either completely or nearly completely buried in the protein interior (Fig. S1C). Mutant OTC proteins in all study subjects were predicted to be less stable than the wild type by the machine learning method (Fig. S2A), whereas force field calculations predicted that 41 mutant proteins found in asymptomatic females and 30 mutant proteins found in females with late onset OTCD would be more stable than the wild type (Fig. S2B). Additionally, five mutant OTC found in males with neonatal onset OTCD, four mutant proteins found in males with late onset OTCD and one mutant found in an asymptomatic male were predicted to be more stable than the wild type (Fig. S2B). Tools such as SIFT and PolyPhen2 that have been used in genomics studies to predict effects of sequence variants on protein function rely on protein conservation and force field calculations based on existing three-dimensional structure (Cheng et al., 2006; Ding and Dokholyan, 2006; Yin et al., 2007a, b). Tradeoffs where increased protein stability reduces enzyme activity have been experimentally explored in T4 lysozyme by Shoichet and colleagues (Shoichet et al., 1995). This leads to experimentally testable hypotheses regarding changes in enzyme stability for these mutations. Most missense mutations found in study subjects were correctly predicted to affect function of the OTC by both SIFT and PolyPhen2; however, amino acid replacements R26Q, D41G, A135E, E239D and A152V were not predicted to affect OTC function by either tool (Fig. S3). Computational tools to evaluate the effect that sequence variants have on protein function are still not reliable, but improvements in this area are necessary as the ability to detect new *OTC* variants will increase with the adoption of genomics approaches.

Neuropsychological Outcomes in Patients with OTCD

In addition to disease onset, the disease severity in participants of the longitudinal study of the OTCD was evaluated using results of neuropsychological tests, number of hyperammonemic

episodes and biomarkers of liver dysfunction. Most female study subjects have average or above average IQ (Fig. 6A). In male participants, earlier disease onset is associated with poorer neurocognitive outcomes (Fig. 6B). These data are consistent with the recent report of neurocognitive outcomes in patients with urea cycle disorders, including OTCD (Seminara et al., 2010; Weisbren et al., 2014).

Hyperammonemic Episodes in Patients with OTCD

Because declining neurocognitive outcomes correlated with the number of hyperammonemic episodes (Weisbren et al., 2014), we examined whether the type of mutation in study subjects correlated with the number of hyperammonemic episodes they experience. The majority of study subjects, who were grouped by gender and disease onset, did not report illness or hyperammonemic episodes during their participation in the longitudinal study (Fig. 7A–C). Mutations found in study subjects were classified as loss of function (LOF), which includes small and large deletions of the parts or entire *OTC* gene, nonsense mutations and sequence changes within consensus splice sites, amino acid replacements and variants in *OTC* introns, which presumably decrease efficiency but do not abolish splicing of the *OTC* mRNA. There was no correlation between the number of hyperammonemic episodes and either the type of mutation or the length of participation in the study among males with neonatal OTCD who did not receive a liver transplant (Fig. 7D). Examples from this group include two study subjects with LOF mutations, each of whom only had one hyperammonemic episode, while a study subject with what is considered a hypomorphic *OTC* allele had the most episodes (Fig. 7D). The number of hyperammonemic episodes in male study subjects with late onset OTCD due to amino acid replacements correlated with the length of their participation in the longitudinal study (Fig. 7E). One male participant with mutation in the consensus splice site, which is considered to be a LOF

mutation, and two study subjects with mutations in *OTC* introns experienced no hyperammonemic episodes (Fig. 7E). The patterns of correlation between the number of hyperammonemic episodes and the length of participation in the study differed in male study subjects with neonatal and late onset OTCD; this could be because males with neonatal OTCD, which is considered to be more severe than late onset disease, leave the study before they have a chance to experience many hyperammonemic episodes. Female study subjects with late onset OTCD due to amino acid replacements and LOF mutations also tended to have more hyperammonemic episodes that correlated with the length of participation (Fig. 7F).

Liver Damage in Patients with OTCD

Some patients with OTCD experience liver damage evidenced by elevated plasma alanine aminotransferase (ALT) and prolonged coagulation time resulting in increased international normalized ratio (INR) (Burlina et al., 2006; Mustafa and Clarke, 2006; Ihara et al., 2013; Gallagher et al., 2014). Among female study subjects who did not receive liver transplant, two with neonatal onset OTCD, 25 with late onset OTCD and 12 asymptomatic participants had mildly elevated plasma ALT when they joined the study (Fig. 8A). Five female participants with late onset disease had moderately elevated ALT and one asymptomatic study subject had severe elevation of plasma ALT when they joined the study (Fig. 8A). Mildly elevated plasma ALT was also present in three male study subjects with neonatal onset OTCD and six male participants with late onset OTCD when they joined the study (Fig. 8B). Mildly elevated INR was present in four female subjects with late onset OTCD and one asymptomatic female while one female with late onset OTCD and one asymptomatic female had moderately elevated INR when they joined the study (Fig. 8C). Two male study subjects with neonatal onset disease had mildly elevated INR and one male with late onset OTCD had moderately elevated INR when they joined the

study (Fig. 8D). None of the patients described here had liver transplants. When both baseline ALT and INR were measured, study subjects who had elevated plasma ALT when they joined the study did not have elevated INR and *vice versa*. In females with symptoms of OTCD, mildly to moderately elevated baseline INR and plasma ALT was present in 3.3% (1 out of 30) and 36.7% (11 out of 30) of participants with LOF mutations, respectively, and 6.8% (4 out of 59) and 40.7% (24 out of 59) of study subjects with missense mutations, respectively (Fig. S4A and B). In asymptomatic female study subjects, elevated plasma ALT at baseline was present in 18.8% (3 out of 16) and 16.9% (11 out of 65) of participants with LOF mutations and amino acid replacements, respectively, while 4.7% (2 out of 43) of participants with amino acid replacements had elevated INR at baseline (Fig. S4C and D). Mildly to moderately elevated INR and mildly elevated plasma ALT at baseline were found in 12.0% (3 out of 25) and 30.9% (13 out of 42) male study subjects with OTCD due to amino acid replacements (Fig. S4E and F). Asymptomatic male study subjects had normal INR and plasma ALT when they joined the study. Subjects with severe (LOF) or mild (amino acid replacements) OTCD showed similar elevations in INR and plasma ALT, suggesting that factors other than the type of *OTC* allele determine whether OTCD will be accompanied with liver dysfunction. Additionally, male and female study subjects had similar incidences of OTCD with mildly elevated plasma ALT (30.9% and 33.9%, respectively), suggesting that both genders are equally susceptible to liver dysfunction associated with OTCD. If liver damage is cumulative, and females with OTCD live longer, they may be at greater risk for detectable liver damage as they age.

A number of participants in the longitudinal study of OTCD had normal liver function when they joined the study but subsequently developed symptoms of hepatic dysfunction. Most study subjects with symptoms of liver dysfunction had mildly elevated plasma ALT (Table S5).

One male study subject with neonatal onset OTCD, five female participants with late onset OTCD and three asymptomatic female participants had moderately elevated plasma ALT at least once during their participation in the study (Table S5). Four study subjects, a male with neonatal onset OTCD and three females with late onset OTCD, had severely elevated plasma ALT at least once during their participation in the study (Table S5). A majority of study subjects who experienced elevated INR al least once during participation in the study had moderately elevated INR, while one female participant with late onset OTCD and three asymptomatic females had severely elevated INR (Table S6). A number of study subjects, one male and two females with neonatal onset OTCD, five males and eight females with late onset disease and two asymptomatic females, had both elevated INR and plasma ALT during their participation in the study. Some study subjects with most elevated markers of liver dysfunction had hyperammonemic episodes while others did not. A male study subject with neonatal onset OTCD who had severely elevated ALT and moderately elevated INR experienced two hyperammonemic episodes during two years of participation in the study. Of the three female study subjects with late onset OTCD and severely elevated plasma ALT, one had moderately elevated INR and experienced one hyperammonemic episode in four years of participation in the study, one had normal INR and one hyperammonemic episode in five years and one had normal INR and no hyperammonemic episodes during five years of her participation in the study. Of the four female study subjects with severely elevated INR, one had no hyperammonemic episodes during five years of her participation in the study and other three are asymptomatic carriers of *OTC* mutations. This suggests a complex relationship between health of the liver and its ability to maintain normal ammonia levels in the blood.

MOLECULAR DIAGNOSTIC STRATEGIES

OTCD is presently most often diagnosed by sequencing 10 *OTC* exons and their intron/exon boundaries due to the non-invasive nature of collecting leukocytes for DNA isolation and improvement and decreasing costs of sequencing technology. Advances in comparative genome and oligonucleotide hybridization techniques led to improvements and increases in diagnosing OTCD due to large deletions, duplications or rearrangements in the *OTC* gene (Arranz et al., 2007; Jakubiczka et al., 2007; Deardorff et al., 2008; Wong et al., 2008; Shchelochkov et al., 2009; Balasubramaniam et al., 2010; Quintero-Rivera et al., 2010). With these methods, OTCD can be confirmed at molecular level in 80%–90% of patients with clinical symptoms of the disease (Yamaguchi et al., 2006; Shchelochkov et al., 2009). As the use of next-generation sequencing becomes standard practice, we expect additional disease-causing mutations will be found in previously unexamined *OTC* gene regulatory regions as well as in the *OTC* introns enabling confirmatory molecular diagnoses for most, if not all, patients with clinical symptoms of OTCD. Another consequence of the wide use of whole exome and genome sequencing will be identification of *OTC* sequence variants in patients who may or may not have clinical symptoms of OTCD.

FUTURE DIRECTIONS

As sequencing of whole exomes and genomes becomes common clinical practice, *OTC* variants will be found in patients who do not and may never present with symptoms of OTCD. Interpretation of this clinically important incidental information is relevant because mutations in the *OTC* gene put patients at risk of developing hyperammonemia, which can have devastating consequences if left untreated, and are associated with nonverbal learning disability (Gyato et al., 2004; Kim et al., 2014), appear to be associated with liver dysfunction (Burlina et al., 2006; Mustafa and Clarke, 2006; Ihara et al., 2013; Gallagher et al., 2014), and have implications for

family planning. Bioinformatic tools can be used to evaluate whether sequence variants in introns either disrupt splicing through creating new splice sites or decrease splicing efficiency of *OTC* mRNA. Predicting effects of sequence variants in regulatory regions of the *OTC* gene requires detailed understanding of the regulation of expression of the *OTC* gene. Enzymatic activity assays have been performed from livers obtained after transplantation, from deceased patients, and biopsy tissue; however, these typically are from liver homogenates with only a few examples where OTC was purified (Snodgrass, 1968; Pierson et al., 1977; Kalousek et al., 1978). Biochemical characterization of human OTC has been relatively limited as numerous factors can confound interpretation. Levels of normal OTC fluctuate with dietary protein intake, illness, and other environmental changes (Klein et al., 2008; McGuire et al., 2013; McGuire et al., 2014). We have previously characterized several OTC mutations based on the enzyme kinetics of purified recombinant human protein expressed in *E. coli* (Morizono et al., 1997a; Morizono et al., 1997b). Limitations of this approach were poor purification efficiencies for some mutant proteins. An affinity column with an immobilized bisubstrate analog of carbamylphosphate and ornithine, N-(phosphonoacetyl)-L-ornithine (PALO) was used. For mutations that affect substrate binding, the PALO affinity column approach was not effective. Simply using affinity tags does not discriminate between soluble trimers and small OTC aggregates that normally occur even when expressing wild type OTC. Expression, purification and characterization of mutant OTC from bacteria are very labor intensive. Enzyme activities have been measured from crude mammalian cell culture lysates in which mutant or wild type OTC was expressed (Nishiyori et al., 1997; Kogo et al., 1998; Augustin et al., 2000; Kim et al., 2006). These approaches can sometimes provide additional information such as *OTC* mutations that affect mitochondrial targeting and processing (Mavinakere et al., 2001) but are inappropriate for obtaining detailed enzyme kinetics.

High throughput functional assays *in vitro* or in cultured cells will still be needed to confirm and validate predicted effects of detected variants. Advances in genome editing will provide the ability to engineer mutations and examine their effects *in vivo* using animal models. The current computational approaches are imperfect, but help identify regions in the protein and mutations that may benefit from further functional characterization. Mutations in which the *in silico* predictions differ from experimental measurements can provide the basis for generating new hypotheses about the structure and function relationships within OTC, and also help with refining prediction algorithms.

Another challenge associated with finding *OTC* sequence variants, found using standard or next generation sequencing methods, is prediction of severity of disease in people with those sequence changes. We have documented that most participants with OTCD enrolled in the longitudinal study did not experience episodes of elevated plasma ammonia whereas some had dozens of hyperammonemic episodes. Additionally, there was no correlation between type of mutation and severity of disease measured by either number of hyperammonemic episodes or liver dysfunction in patients with late onset OTCD. This suggests that complex relationship between genetic and environmental factors affects disease outcome and better understanding of these factors can improve our ability to avoid devastating consequences of hyperammonemia. Genome wide association studies (GWAS) could be used to identify genetic modifiers of OTCD but the small number of patients available for such studies will require development of new computational and statistical methods to analyze the data. International collaborations among consortia that study natural histories of OTCD and other urea cycle disorders (Seminara et al., 2010; Batshaw et al., 2014; Summar et al., 2014) will help overcome the limited number of subjects available for such GWAS. These studies of the natural history of OTCD are also likely

to identify environmental factors that contribute to the variability of OTCD presentation. Encouraging the development of international patient registries and standardization of reporting will be essential next steps in refining our understanding of factors that contribute to the severity of rare diseases such as OTCD.

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Figure Legends

Fig. 1. Schematic of a hepatocyte highlighting the various enzymes, transporters, and metabolites of the urea cycle.

Shown in yellow is a mitochondrion containing the proximal enzymes N-acetylglutamate synthase (NAGS), carbamylphosphate synthetase 1 (CPS1), ornithine transcarbamylase (OTC), and the ornithine transporter (ORNT) and citrin transporters. Citrulline is transported out of the mitochondrion, and converted by argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), and arginase I (ARG1) to produce urea and regenerate ornithine that is reutilized by OTC.

Fig. 2. Missense and nonsense mutations in *OTC* and their overlap with CpG dinucleotides.

The number of different missense and nonsense mutations that cause OTCD was plotted against their codon number. Blue symbols, missense mutations. Orange symbols, nonsense mutations. Gray bars, codons that overlap with CpG dinucleotides on sense and antisense strands.

Fig. 3. Conservation and solvent accessible area of amino acids affected by missense mutations in patients with OTCD.

A: Conservation scores of amino acids affected by missense mutations found in females (green, n = 73) and males with either neonatal (red, n = 66) or late onset (blue, n = 57) OTCD. **B:** Frequency distribution of conservation scores of the amino acids affected by missense mutations that cause OTCD. **C:** Solvent accessible areas of amino acids affected by missense mutations found in females and males with either neonatal or late onset OTCD. **D:** Frequency distribution of the solvent accessible areas of amino acids affected by missense mutations that cause OTCD. **E–G:** Scatter plots of conservation scores *vs.* solvent accessible areas of amino acids affected by

mutations that cause OTCD in female patients (**E**) and male patients with either neonatal (**F**) or late (**G**) onset OTCD.

Fig. 4. Destabilization of the OTC protein by amino acid replacements found in patients with OTCD.

A: Calculated difference between stability of mutant and wild type OTC using support vector machines. **B:** Calculated difference between wild type and mutant OTC using force fields. **C:** Frequency distribution of $\Delta\Delta G$ values calculated using support vector machines. **D:** Frequency distribution of $\Delta\Delta G$ values calculated using force fields. Red, males with neonatal onset OTCD (n = 87). Blue, males with late onset OTCD (n = 70). Green, females with OTCD (n = 95)

Fig. 5. Recurrent mutations found in participants enrolled in the longitudinal study of OTCD.

Number of study subjects with each missense and nonsense mutation and their overlap between affected codons and CpG dinucleotides on sense and antisense strands of the *OTC* coding region were plotted for each codon of the *OTC* open reading frame. Blue symbols, positions of missense and nonsense mutations in the OTC coding sequence. Gray bars, codons that overlap with CpG dinucleotides on sense and antisense strands.

Fig. 6. Neurocognitive outcomes in female and male participants of the longitudinal study of OTCD.

A: IQ scores of the neonatal onset females (dark purple), late onset females (light purple), asymptomatic females (lavender) study subjects. **B:** IQ scores of the neonatal onset males (navy),

late onet males (light blue), asymptomatic males (cyan) study subjects. Error bars represent median and interquartile ranges. Normal IQ ranges are shown in grey.

Fig. 7. Hyperammonemic and illness episodes experienced by the participants in the longitudinal study of the OTCD.

Distribution of reported illness (gray) and hyperammonemic (cyan) episodes by the male study subjects with either neonatal onset (**A**) or late onset (**B**) OTCD and female study subjects with late onset OTCD (**C**). Frequency of hyperammonemic episodes reported by male study subjects with either neonatal onset (**D**) or late onset (**E**) OTCD and female study subjects with late onset OTCD (**F**) due to missense (blue), loss of function (orange) or intronic (magenta) mutations.

Fig. 8. Biomarkers of liver damage in participants of the longitudinal study of OTCD.

Baseline values of plasma ALT in female (**A**) and male (**B**) study subjects; baseline INR values in female (**C**) and male (**D**) study subjects. Plasma ALT activities of 35–105 U/L and 40–120 U/L were considered mildly elevated in females and males, respectively. Plasma ALT activities of 105–175 U/L and 120–200 U/L were considered moderately elevated in females and males, respectively. Plasma ALT activities above 175 and 200 U/l were considered severely elevated in females and males, respectively. INR values between 1.2 and 1.5 were considered mildly elevated, while values between 1.5 and 2.5 were considered moderately elevated. Gray areas indicate normal ranges of plasma ALT and INR values. Neonatal onset females (dark purple), late onset females (light purple), asymptomatic females (lavender), neonatal onset males (navy), late onset males (light blue), asymptomatic males (cyan)

Tables

Table 1. Types of mutations that cause OTCD.

Mutation type	**Number of mutations**
Missense	264
Nonsense	39
Frame shift	40
In frame indels	11
Splice site errors	60
Extending[a]	2
Regulatory	1
Disease presentation	
Neonatal	163
Late	87
Female	144
No information	23
Total	**417**
New mutations identified through UCDC[b]	23

[a]Extending mutations are sequence changes within the OTC translation termination codon that extend the *OTC* open reading frame and protein. [b]UCDC, Urea Cycle Disorders Consortium.

Table 2. Common polymorphisms in *OTC*. [Table 2 is in landscape orientation, please refer to separately uploaded RevisedTable 2.docx]

Table 3. Disease onset and the type of defect in the *OTC* gene in patients enrolled in the longitudinal study of OTCD.

Disease onset	**Gender**		**OTC defect**		
			Loss of function	**Amino acid replacement**	**Variant in introns**
Neonatal	Male	32	7	22	3
	Female	5	2	3	0
Late	Male	36	1	33	2
	Female	92	29	62	1
Asymptomatic	Male	6	0	6	0
	Female	88	17	70	1

SUPPLEMENTARY DATA

Figure 1

Fig. 1

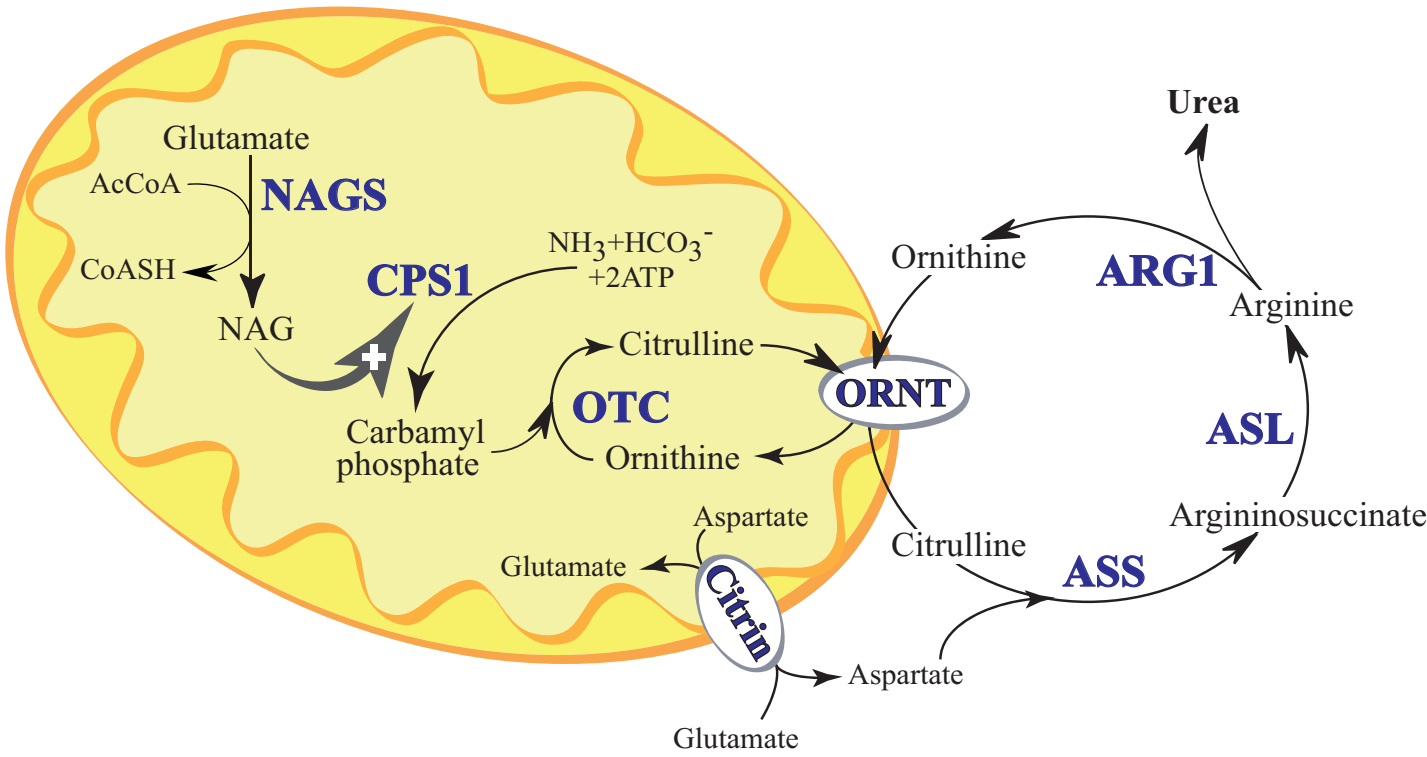

Figure2

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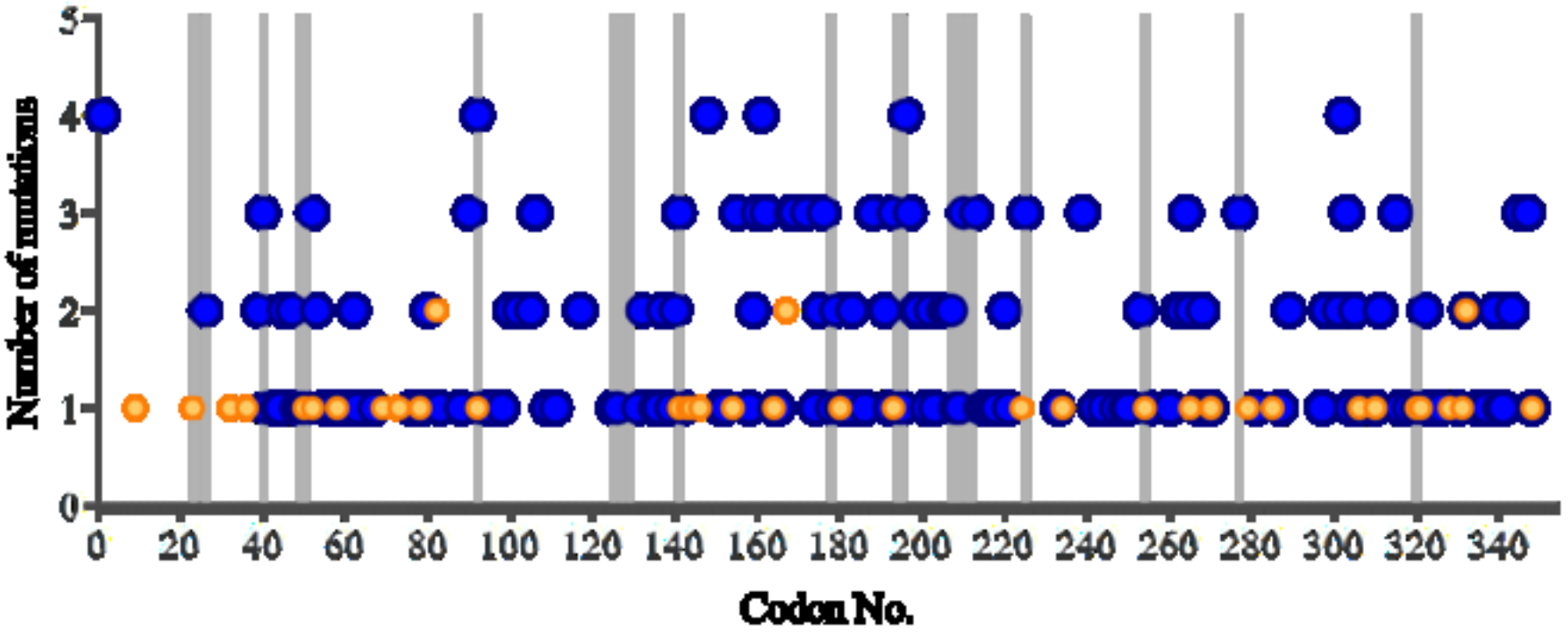

Figure 3
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Fig. 3

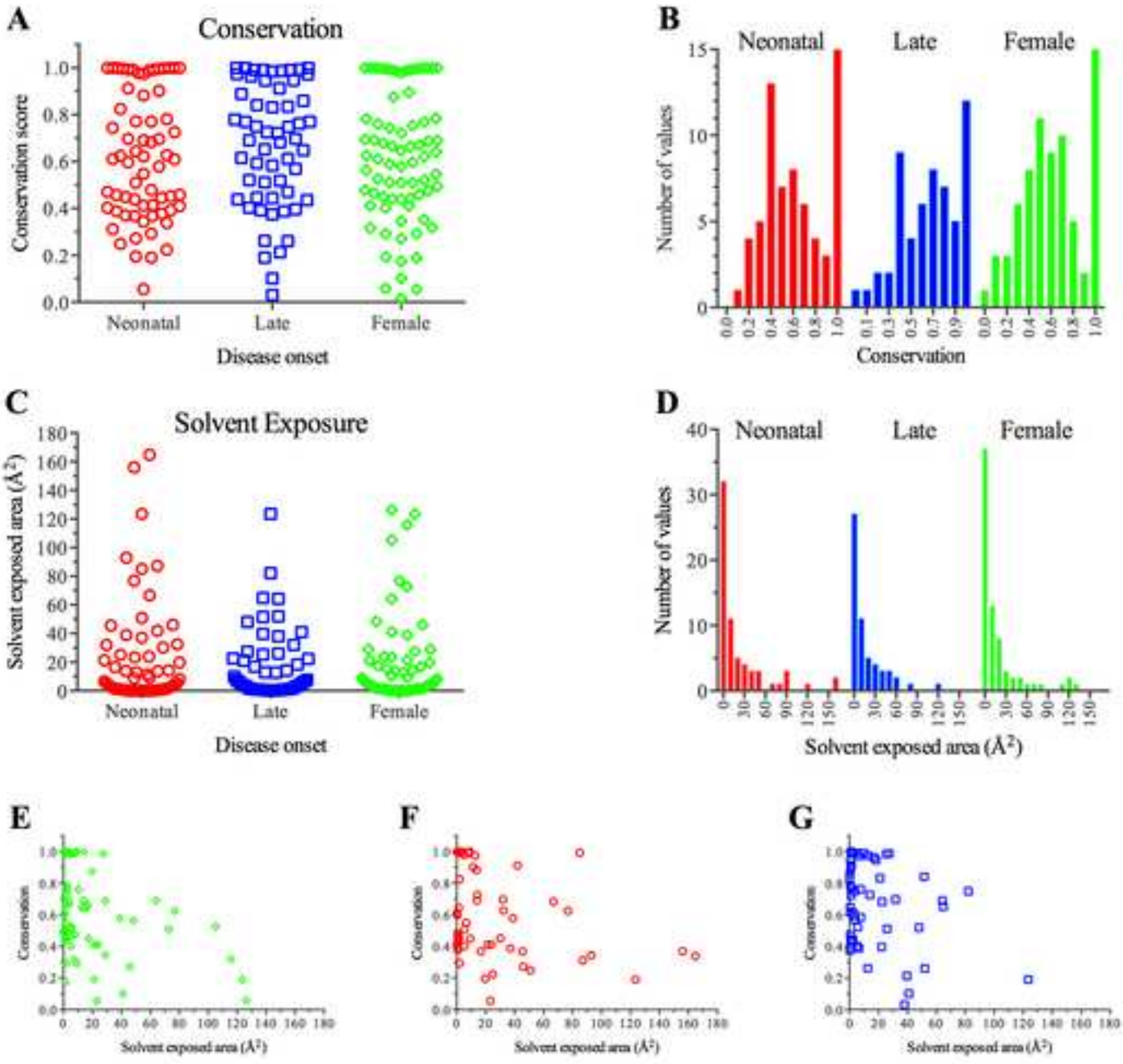

Figure 4
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Fig. 4

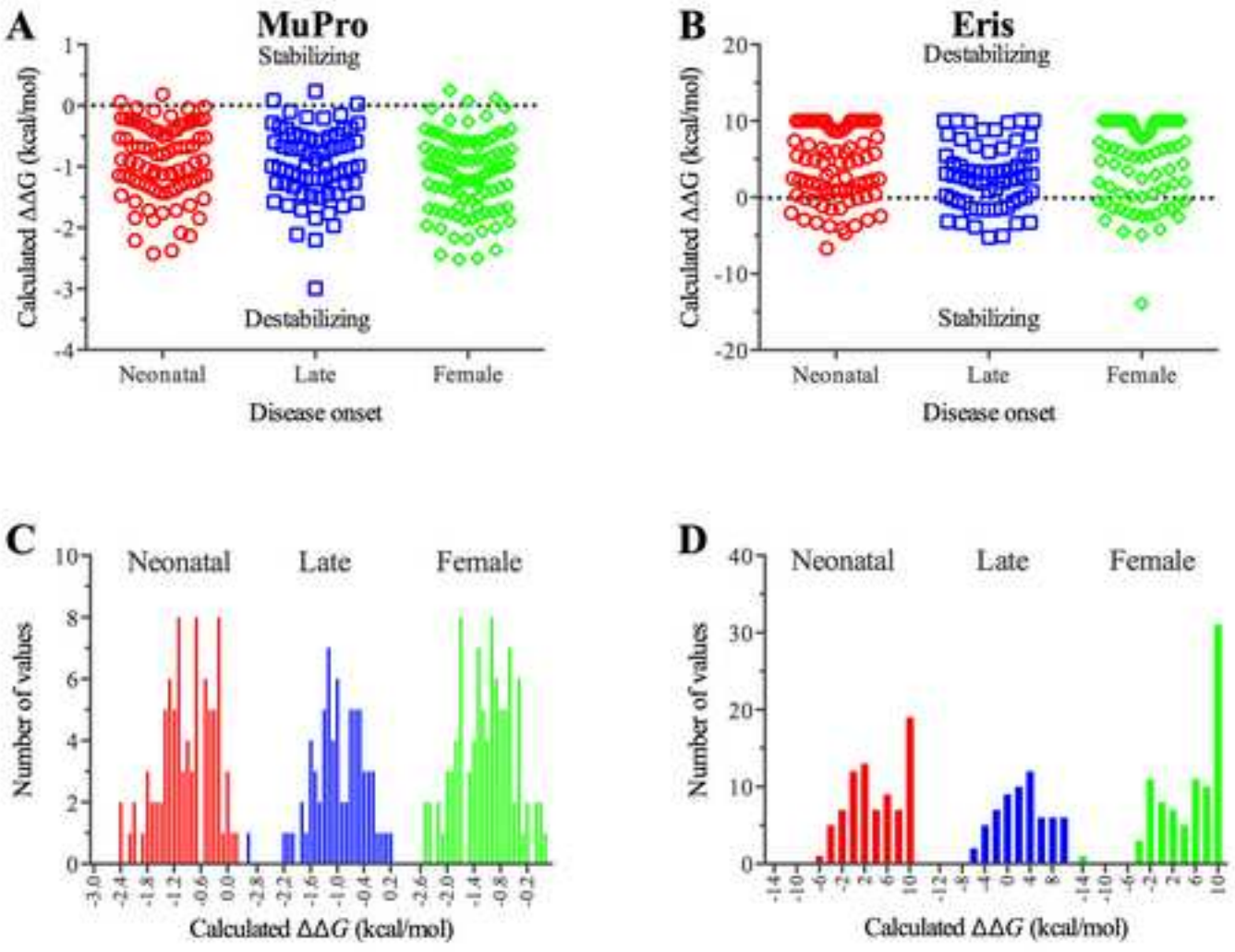

Figure5

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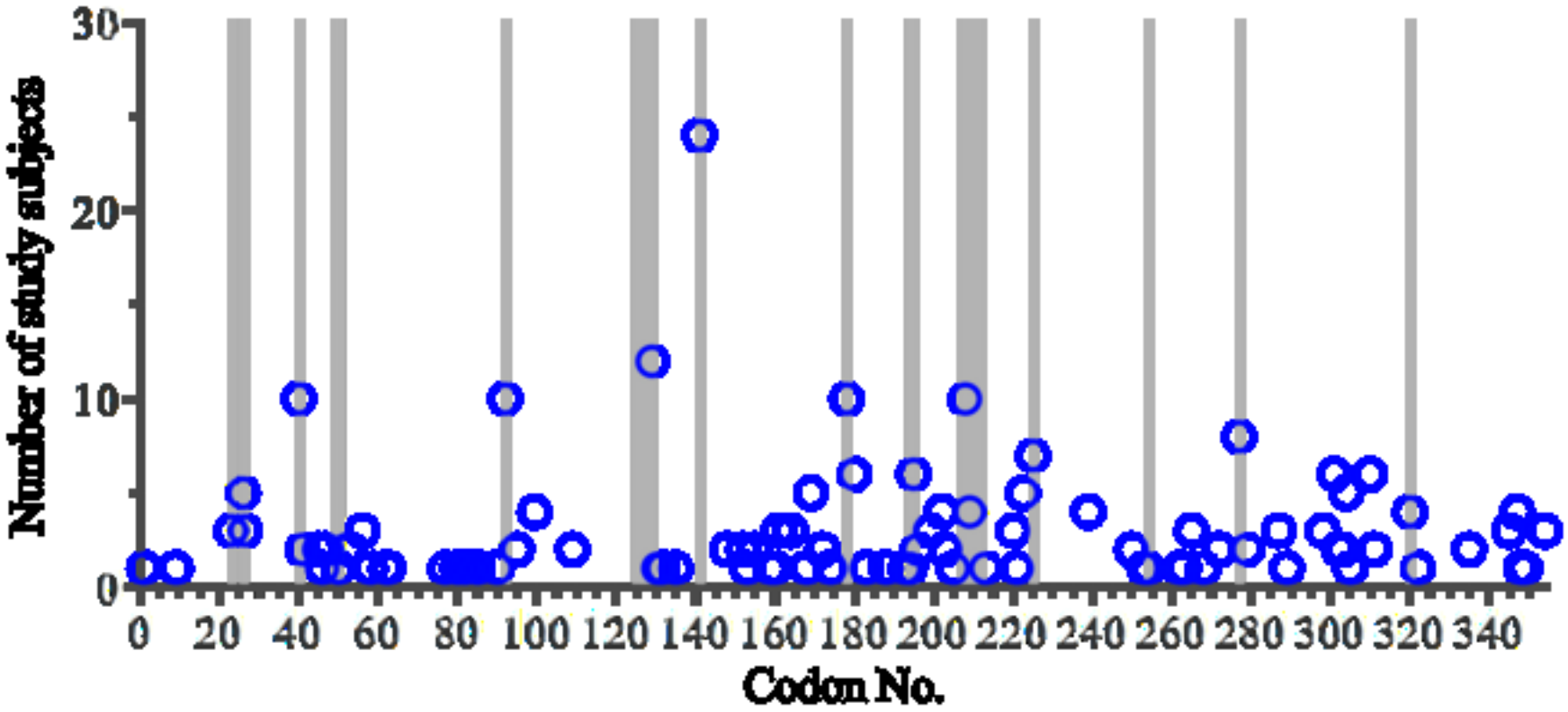

Figure 6
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Fig. 6

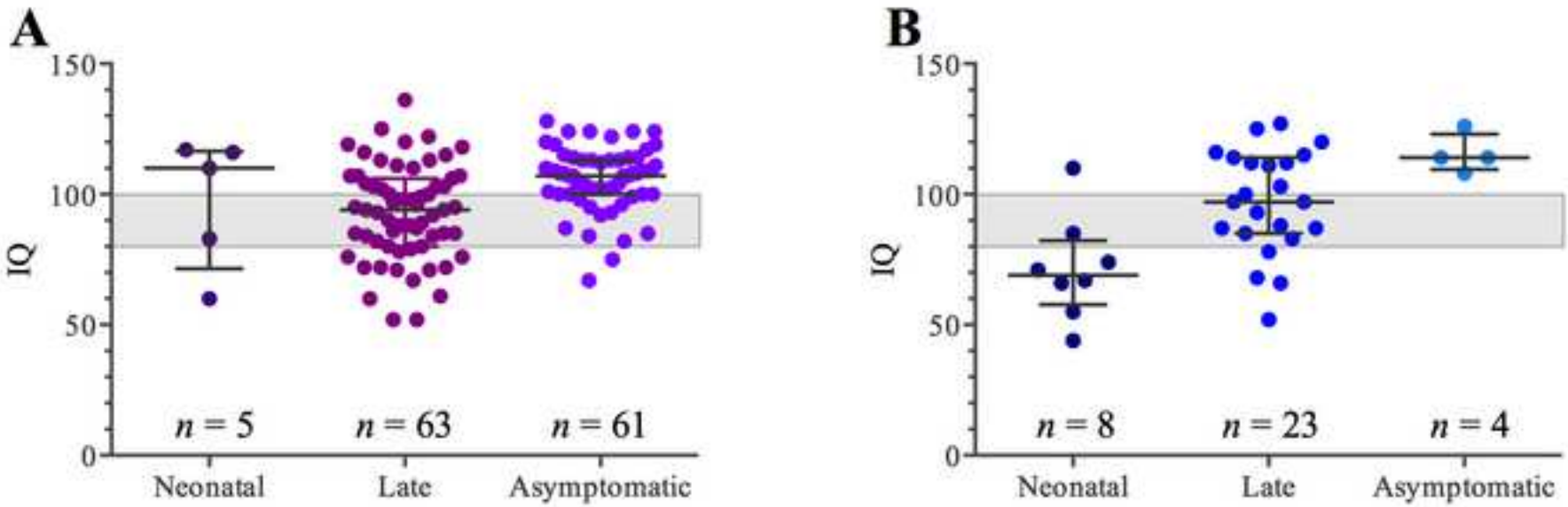

Figure 7
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Fig. 7

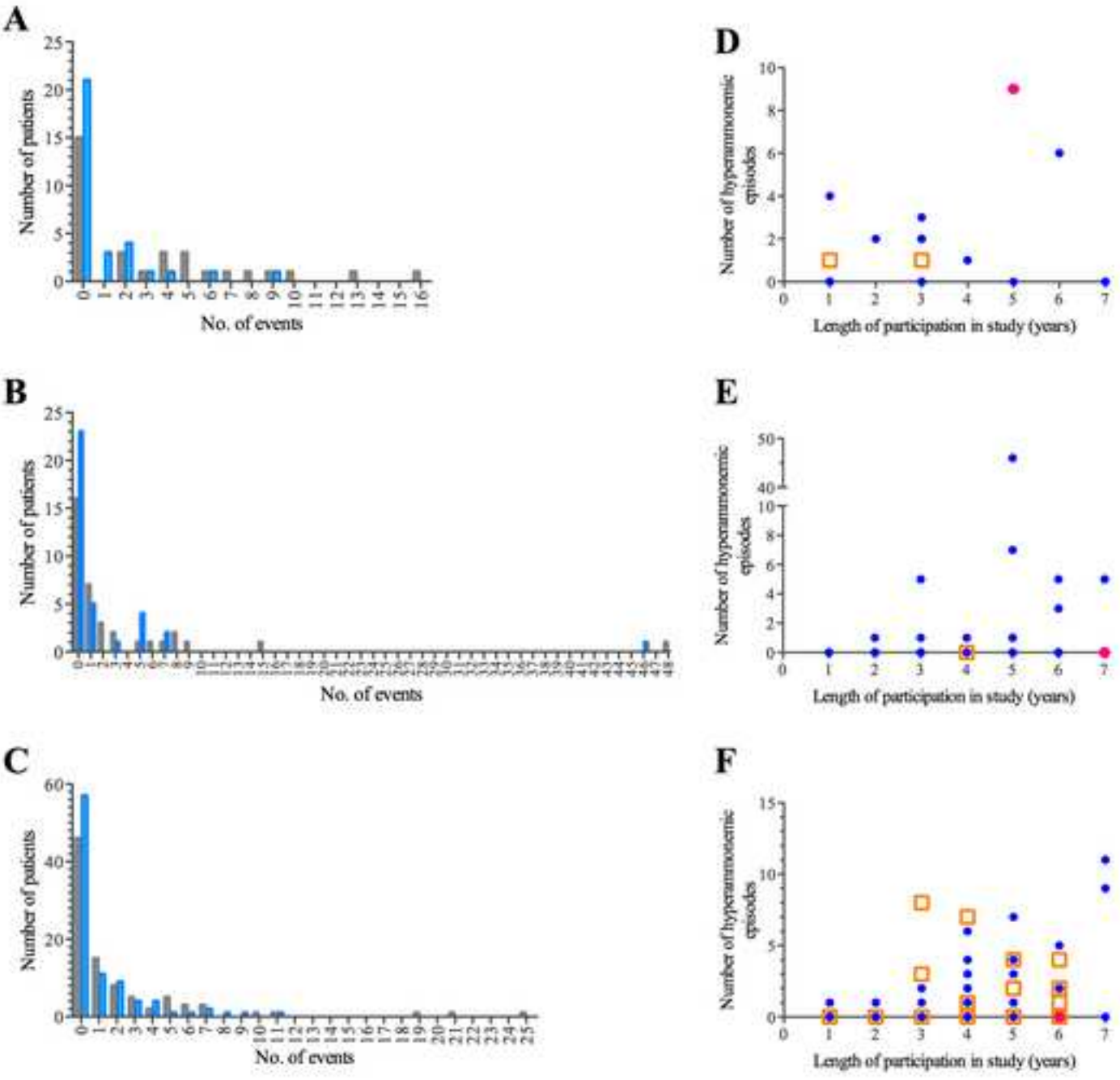

Figure 8
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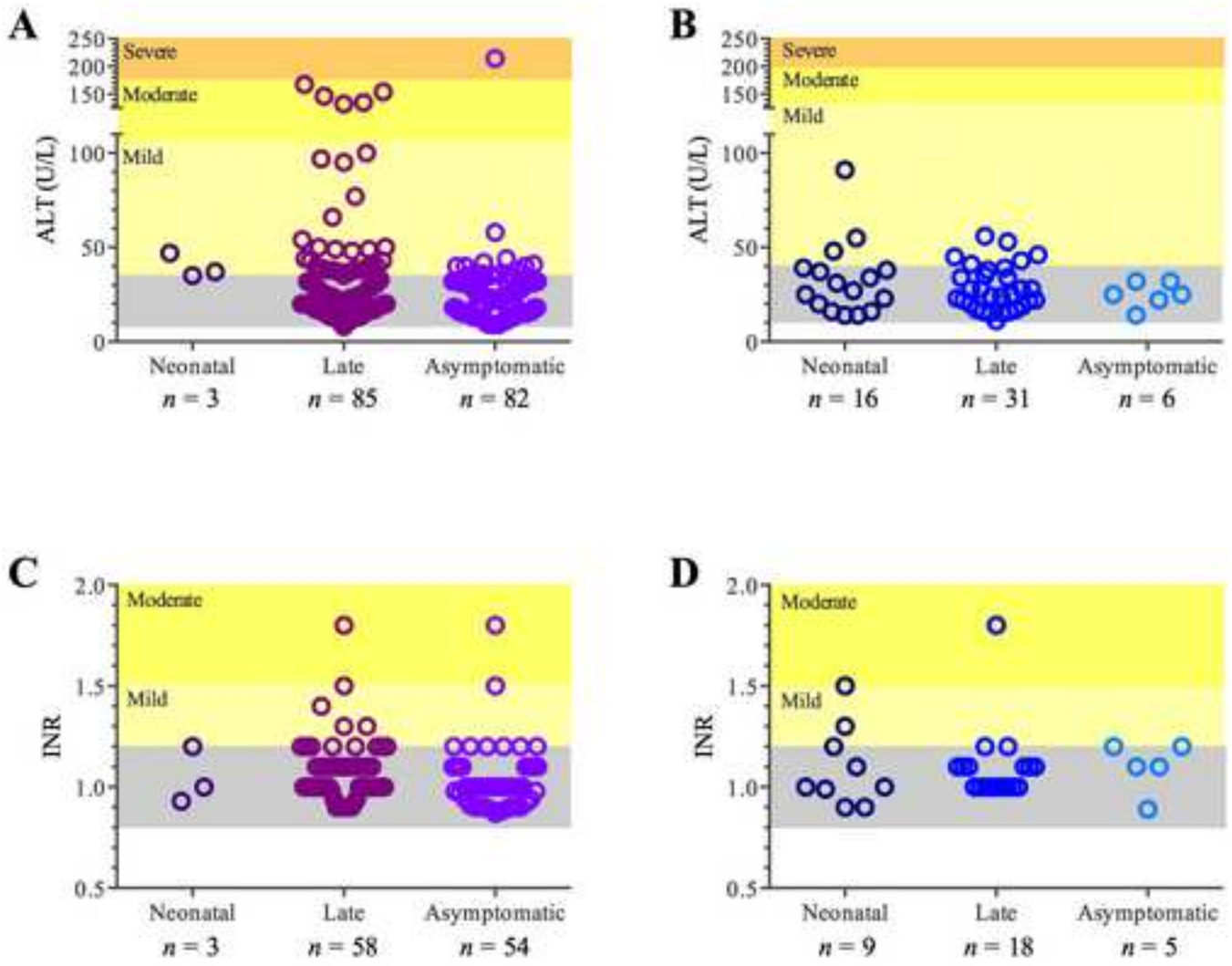
Fig. 8



Table

Table 2. Common polymorphisms (MAF > 1%) in the upstream regulatory region, exons and introns of the human *OTC* gene.

No.	Region	Nucleotide/amino acid change/SNP ID	Allele frequency	Reference/submitter
1.	Upstream region	c.–520_–518delCT rs200016637	CTTCTT: 36.88% (610/1659); CTT: 63.12% (1049/1659)	1000 genomes[a]
2.	Upstream region	c.–514_–511delTCTA rs201351170	TCTATCTA: 36.09% (600/1659); TCTA: 63.91% (1059/1659)	1000 genomes
3.	Upstream region	c.–512insTTCT rs57752938	TTCTTTCT: 28%, 38%, 29%; TTCT: 72%, 62%, 71%[b]	Azevedo et al., 2003
4.	Upstream region	c.–441G>A rs5917572	A: 23%, 38%, 24%; G: 77%, 62%, 76%[b] A: 31.28% (519/1659); G: 68.72% (1140/1659) A: 30.30% (547/1805); G: 68.42 (1235/1805); O: 1.27% (23/1805)	Azevedo et al., 2003 1000 genomes dbSNP build 138[c]
5.	Upstream region	c.–365G>A rs5963030	A: 15%, 16%, 5%; G: 85%, 84%, 95%[b] A: 12.18 (201/1659); G: 87.82% (1458/1659)	Azevedo et al., 2003 1000 genomes
6.	Upstream region	c.–359A>G rs5917573	G: 23%, 31%, 27%; A: 77%, 69%, 73%[b] G: 31.28% (519/1659); A: 68.72% (1140/1659) G: 31.22 (1142/3646); A: 68.68% (2504/3646)	Azevedo et al., 2003 1000 genomes dbSNP build 138
7.	Upstream region	c.–294G>A rs139948134	A: 1.33% (22/1654); G:98.67% (1632/1654)	1000 genomes
8.	Exon 2	c.137A>G p.Arg46Lys rs1800321[d]	G: 32%; A: 68% (71 alleles assayed)[e] G: 28%; A: 72% (123 alleles)[f]; G: 46%; A: 54% (46 alleles)[g] G: 19.70% (327/1659); A: 80.30% (1332/1659) G: 25.24% (2189/8671); A: 72.07% (6249/8671); O[h]: 2.69% (233/8671) G: 29.03% (3066/10560); A: 70.97% (7494/10560)	Plante and Tuchman, 1998 Azevedo et al., 2002a 1000 genomes dbSNP build 138 NHLBI-WES[i]
9.	Intron 3	c.299–39_299–40insT rs148081863 rs143626513	GT: 34%; G: 66% (86 alleles screened) GT: 3.80% (141/3701); G: 96.20% (3560/3701) GT: 11.61% (192/1654); G: 88.39% (1462/1654) GT: 16.37% (1664/10167); G: 83.67% (8503/10167)	Climent and Rubio, 2002a 1000 genomes dbSNP build 138 NHLBI-WES[i]
10.	Intron 3	c.299–17delT rs200509203	T: 0%; TT: 100% (87 alleles screened) T: 1.33% (22/1659); TT: 98.67% (1632/1659)	Climent and Rubio, 2002a 1000 genomes
11.	Intron 3	c.299–8A>T rs73196229	A: 50%; T: 50% (76 alleles)[e] A: 34%; T: 66% (93 alleles)[e] A: 11.60% (192/1659); T: 88.4% (1467/1659) A: 11.76% (195/1658); T: 88.24% (1463/1658) A: 15.65% (1652/10558); T: 84.35% (8906/10558)	Tuchman and Plante, 1995 Climent and Rubio, 2002a 1000 genomes dbSNP build 138 NHLBI-WES
12.	Intron 4	c.387–7G>A[j] rs1800326	G: 29%; A: 71% (45 alleles screened)[e] A: 0%; G: 100% (164 alleles)	Plante and Tuchman, 1998 Azevedo et al., 2002a
13.	Exon 5	c.429T>C p.Tyr143Tyr	C: 0%; T: 100% (51 alleles)[e] C: 2%; T: 98% (59 alleles)[e]	Climent and Rubio, 2002a Tuchman and Plante, 1995

		rs145777402	C: 4.22% (45/10561); T: 95.78% (10516/10561)	NHLBI-WES
14.	Intron 5	c.541–63G>A rs2235125	G: 62%, 71%, 30%; A: 38%, 29%, 70%[b] G: 47.90% (794/1659); A: 52.10% (865/1659) G: 40.37% (1694/4196); A: 54.12% (2271/4196); O: 5.48% (230/4196); N[h]: 0.02% (1/4196)	Azevedo et al., 2003 1000 genomes dbSNP build 138
15.	Intron 7	c.718–14T>C rs55722856	C: 5.13% (85/1657); T: 94.87% (1572/1657) C: 4.90% (82/1659); T: 95.10% (1577/1659) C: 6.92% (731/10561); T: 93.08% (9830/10561)	dbSNP build 138 1000 genomes NHLBI-WES
16.	Exon 8	c.809A>G p.Gln270Arg[k] rs1800328	G: 12.5%; A: 87.5% (56 alleles)[e] G: 4%; A: 96% (169 alleles) G: 2% (34/1659); A: 98% (1625/1659) G: 3.05% (191/6272); A: 96.95% (6081/6272) G: 3.32% (351/10561); A: 96.68% (10210/10561)	Plante and Tuchman, 1998 Azevedo et al., 2002a 1000 genomes dbSNP build 138 NHLBI-WES
17.	Intron 8	c.867+35G>T rs62622415	G: 38%; T: 62% (26 alleles) G: 1.30% (22/1659); T: 98.70% (1637/1659) G: 1.33% (22/1654); T: 98.67% (1632/1654) G: 2.71% (286/10534); T: 97.28% (10248/10534)	Azevedo et al., 2002a 1000 genomes dbSNP build 138 NHLBI-WES
18.	Intron 9	c.1006–70C>T rs12557315	T: 21%, 26%, 0%; C: 79%, 74%, 100%[b] T: 21.50% (356/1659); C: 78.5% (1303/1659) T: 17.40% (731/4201); C: 79.53% (3341/4201); O: 3.07% (129/4201) T: 16.17% (582/3600); C: 83.83% (3018/3600)	Azevedo et al., 2003 1000 genomes dbSNP build 138 NHLBI-WES

[a]Variant frequency reported in the 1000 Genomes Project. The 1000 Genomes Project seeks to provide a comprehensive resource on human genetic variation. [b]Screening was done in healthy males from Portugal (*n* = 85), Czech Republic (*n* = 100–142) and Mozambique (*n* = 40). Not all individuals were screened for all SNPs in the Czech samples. [c]Variant frequency reported in the dbSNP build 138 track of the UCSC Genome Browser. The dbSNP database is curated by the National Center for Biotechnical Informatics containing short genetic variations. Build 138 of the database was used for this table. [d]Reference sequence used is RefSeq NM_000531.3. In RefSeq NM_000531.3 codon 46 encodes a Lys. We more commonly see Arg at this position. [e]Samples were from subjects suspected of OTC deficiency. [f]Screening was done in Portuguese healthy males. [g]Screening was done in healthy males from Mozambique. [h]Nucleotides other than A, T, G and C. N represents any nucleotide; O would normally represent a null allele in a hemizygote, but may also include sequencing errors. [i]Variant frequency reported in the NHLBI Exome Variant Server. The NHLBI-WES contains sequence information from over 200,000 individuals. [j]Annotated as splice site variant in dbSNP 138. [k]This is a commonly seen polymorphism; however, SIFT and Polyphen2 scores for this are deleterious (0.00) and probably damaging (0.977), respectively.

SUPPLEMENTARY DATA

Each site in the Urea Cycle Disorders Consortium (UCDC) (Batshaw et al., 2014) received Institutional Review Board approval for the study and obtained informed consent. Mutations and polymorphisms in the *OTC* gene were collected by querying published literature, the Human Gene Mutation Database, the Leiden Open Variation Database (LOVD) (Fokkema et al., 2011) and 1000 Genomes project (Abecasis et al., 2012) databases, the NHLBI Exome Variant Server (ESP6500SI-V2) (2013) and the UCDC patient registry. The following information was retrieved form the UCDC patient registry: patients' genotype, gender, age, onset of OTCD, whether each patient received liver transplant, neurocognitive test results, number of illnesses and hyperammonemic episodes during participation in the study, and results of plasma alanine aminotransferase and international normalized ratio.

SIFT (Kumar et al., 2009) and PolyPhen2 (Adzhubei et al., 2010) were used to predict possible effects of missense mutations on the function of OTC enzyme. Eris (Ding and Dokholyan, 2006; Yin et al., 2007a, b) and MuPro (Cheng et al., 2006) were used to calculate the difference between stabilities of mutant and wild type OTC proteins. The conservation score of amino acids in the OTC protein sequence was derived from the information entropy of each amino acid in the WebLogo (Crooks et al., 2004) alignment of 566 OTC protein sequences. The solvent accessible surface of amino acids in the OTC trimer was calculated using pdbasa, an implementation of the Shrake and Rupley method by Ho using a point mesh of 9600 (Shrake and Rupley, 1973; Ho, 2014).

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Conservation and solvent accessible area of amino acids affected by missense mutations in patients enrolled in the longitudinal study of OTCD.

A: Conservation scores of amino acids affected by missense mutations in male and female study subjects with neonatal or late onset OTCD or without symptoms of the disease. **B:** Frequency distribution of conservation scores of the amino acids affected by missense mutations that cause OTCD. **C:** Solvent accessible area of amino acids affected by missense mutations in male and female study subjects with neonatal or late onset OTCD or without symptoms of the disease.

Fig. S2. Destabilization of the OTC protein by amino acid replacements found in patients enrolled in the longitudinal study of OTCD.

A: Calculated difference between stability of mutant and wild type OTC using supported vector machines. **B:** Calculated difference between wild type and mutant OTC using force fields.

Fig. S3. Predicted effects of missense mutations on the function of OTC protein in the participants enrolled in the longitudinal study of OTCD.

SIFT (**A**) and PolyPhen2 (**B**) scores of missense mutations found in study subjects of the longitudinal study of the OTCD. Missense mutations with SIFT scores between 0 and 0.05 are considered to affect protein function. Missense mutations with PolyPhen2 scores between 0.85 and 1 are considered to be probably damaging, while missense mutations with scores between 0.2 and 0.85 are considered to be possibly damaging to protein function.

Fig. S4. Biomarkers of liver damage in participants of the longitudinal study of OTCD.

Baseline plasma ALT values in female study subjects with symptoms of OTCD (**A**), asymptomatic female participants (**C**) and male study subjects with OTCD (**E**) due to loss of OTC function (orange), amino acid replacements (blue) and variants in introns (magenta). Plasma ALT activities of 35–105 U/L and 40–120 U/L were considered mildly elevated in females and males, respectively. Plasma ALT activities of 105–175 U/L and 120–200 U/L were considered moderately elevated in females and males, respectively. Plasma ALT activities above 175 and 200 U/L were considered severely elevated in females and males, respectively. Baseline plasma INR values in female study subjects with symptoms of OTCD (**B**), asymptomatic female participants (**D**) and male study subjects with OTCD (**F**) due to loss of OTC function (orange), amino acid replacements (blue) and variants in introns (magenta). INR values between 1.2 and 1.5 were considered mildly elevated, while values between 1.5 and 2.5 were considered moderately elevated. Gray areas indicate normal ranges of plasma ALT and INR values.

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Fig S1

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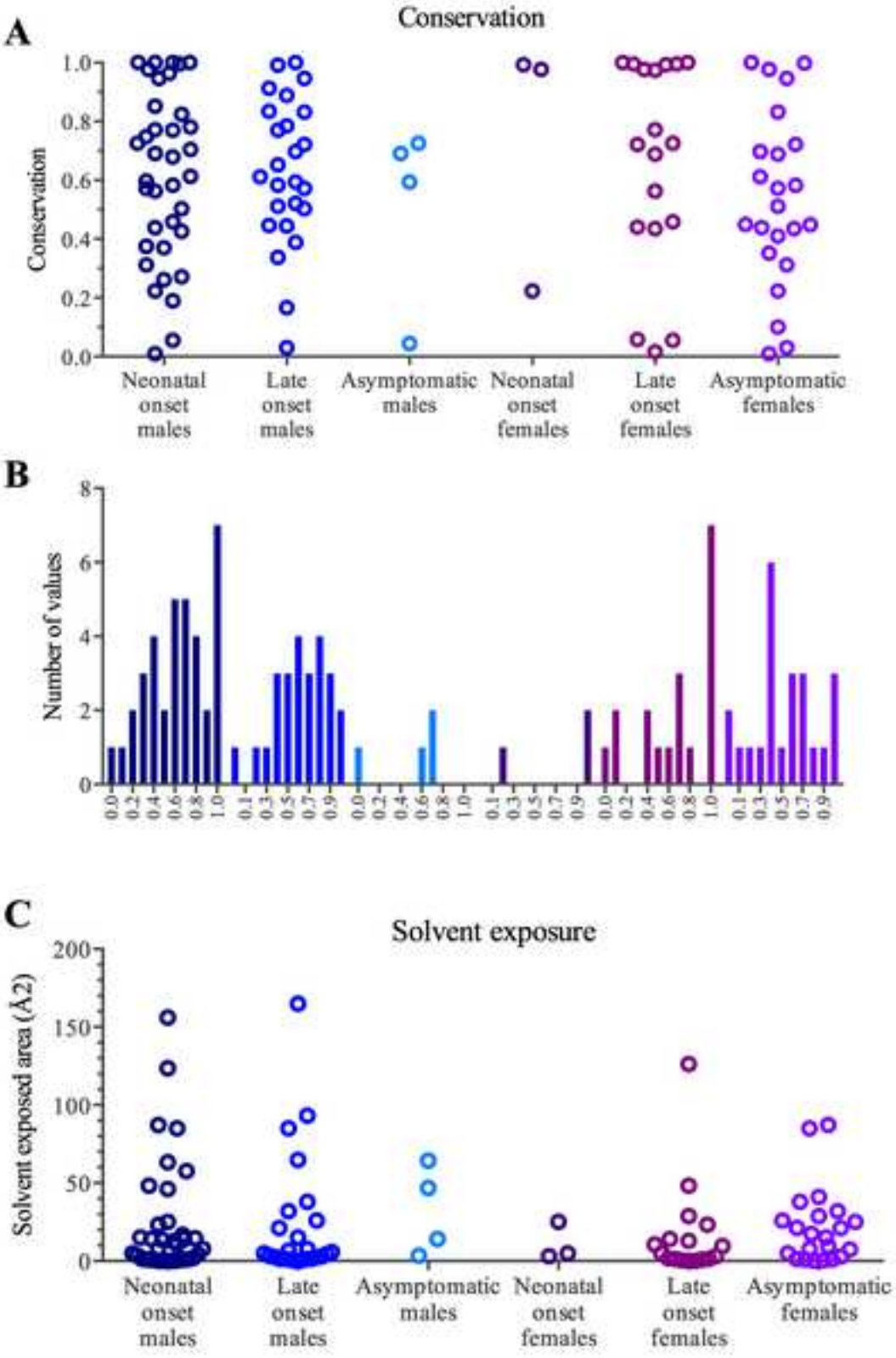

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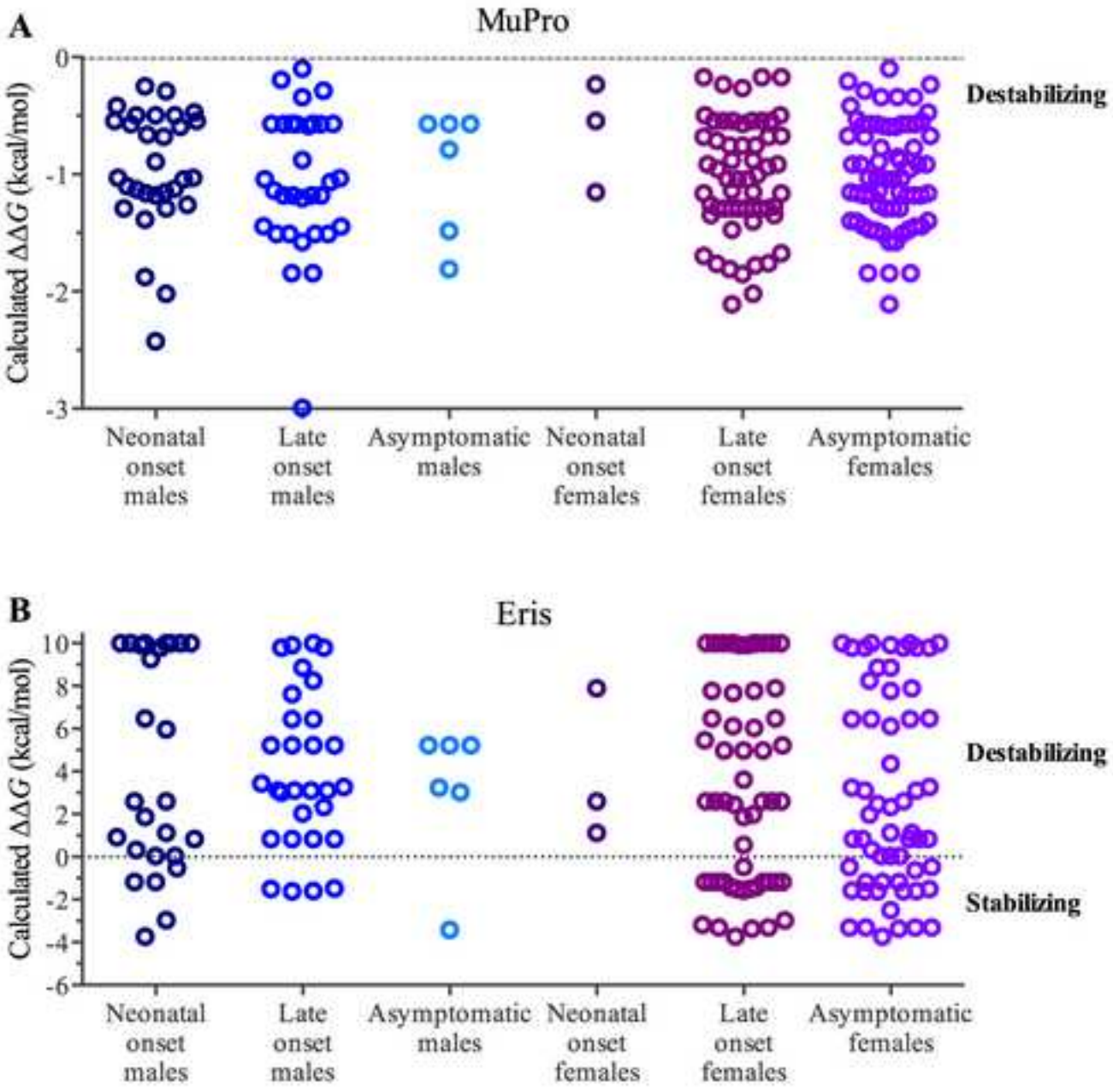

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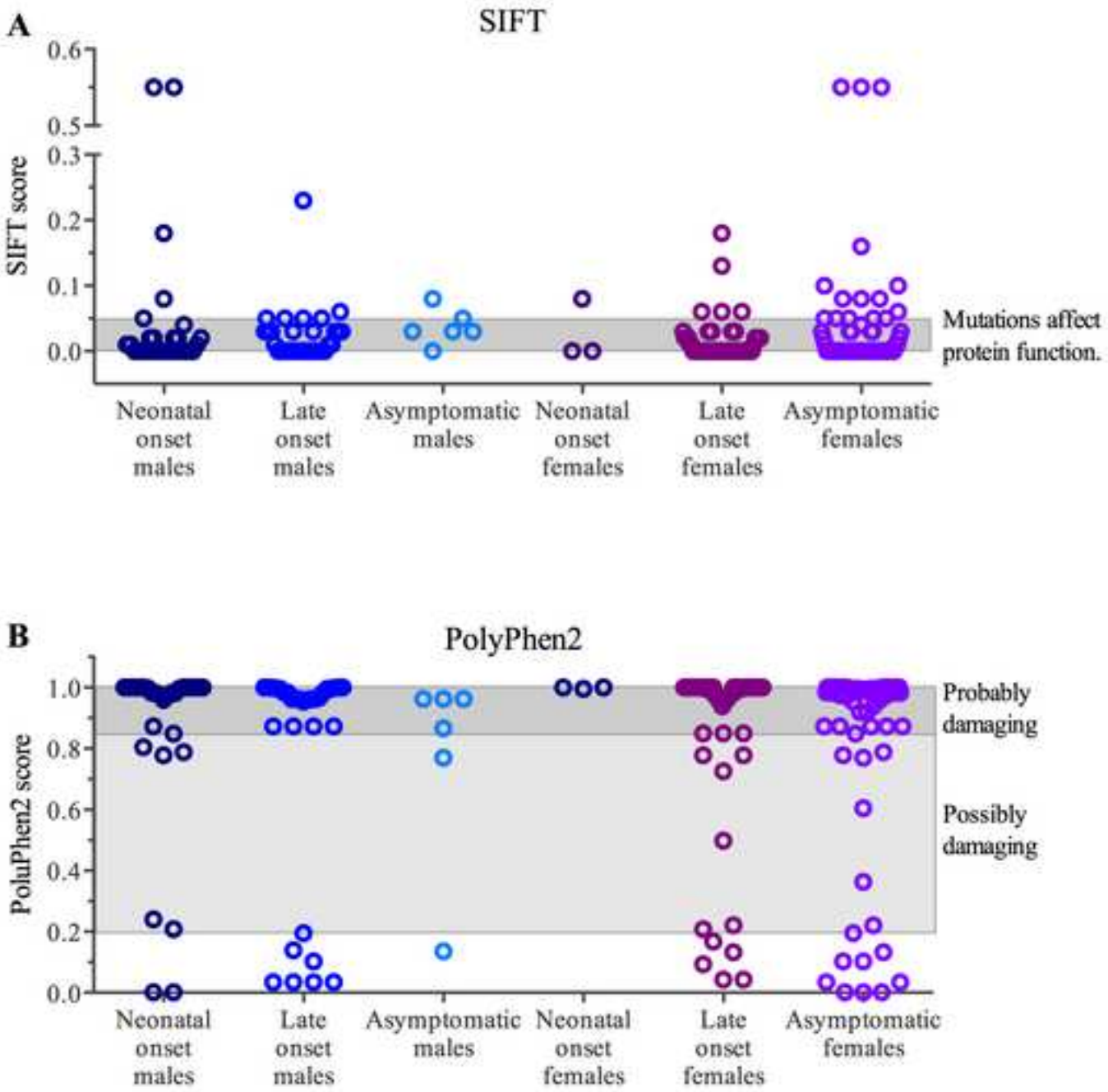

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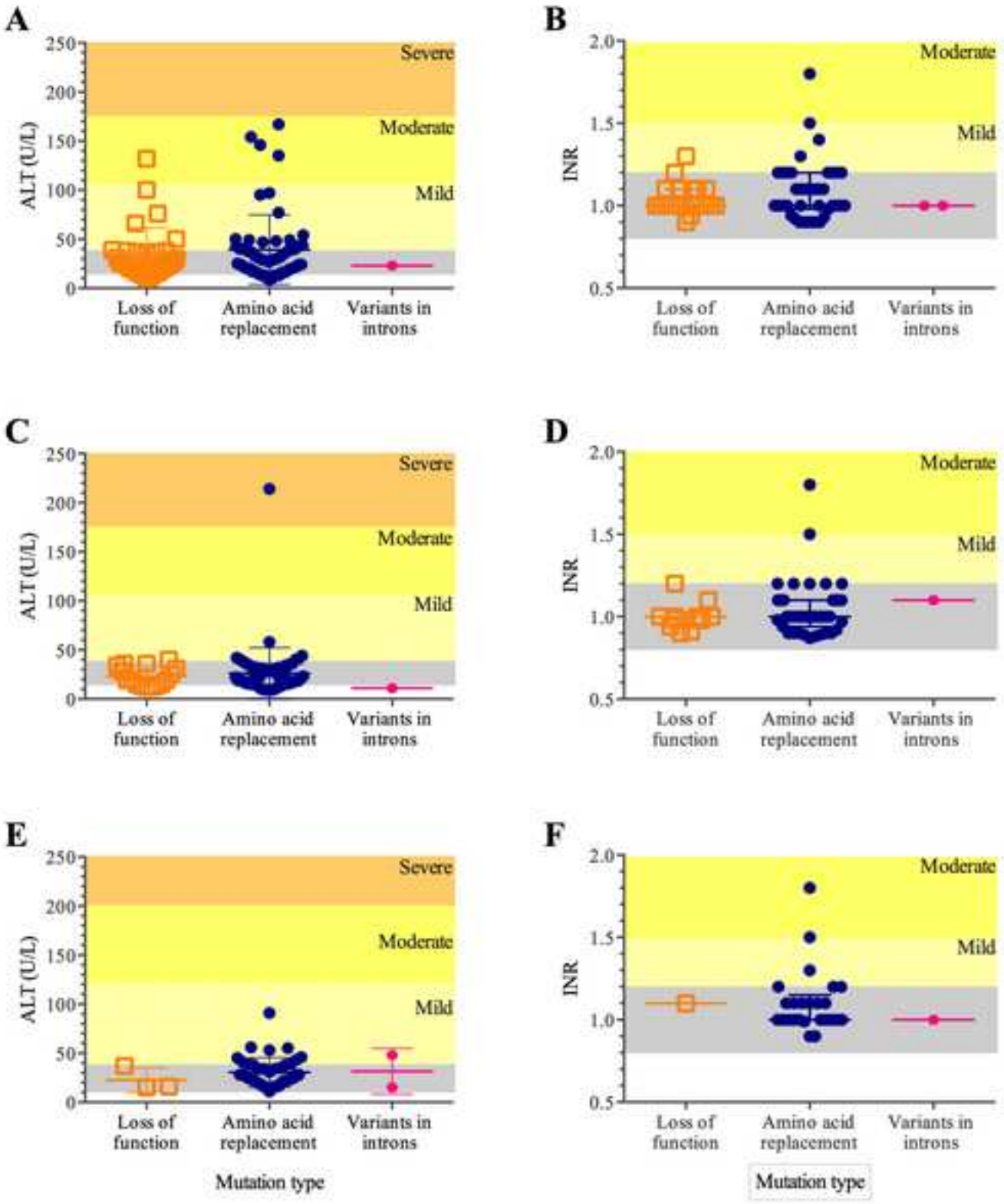

Table S1

Table S1. Mutations in the *OTC* gene associated with OTC deficiency.

No.	Exon	Codon[a]	Nucleotide change[b]	Amino acid or other change	Function	% Enzyme activity /[%] ^{15}N ammonia incorporation[c]	Disease presentation[d]	Reference[e]
1			c.-366A>G		Regulatory		Female	Luksan et al. [2010]
2	Exon 1	1	c.1A>G	p.Met1Val	Missense		Female	Oppliger Leibundgut et al. [1995]
3		1	c.1A>T	p.Met1Leu	Missense		Neonatal	Yamaguchi et al. [2006]
4		1	c.2T>C	p.Met1Thr	Missense		Female	Yamaguchi et al. [2006]
5		1	c.3G>A	p.Met1Ile	Missense		Female	Climent and Rubio [2002b]
6		9	c.25T>G	p.Leu9*	Nonsense		Neonatal	Kim et al. [2006]
7		10,11	c.28_31del AACA	p.Asn10_Asn11	Frameshift		Female	Yamaguchi et al. [2006]
8		10	c.29dupA	p.fsX	Frameshift		Female	Yamaguchi et al. [2006]
9		14	c.40delT	p.Phe14Leufs	Frameshift		Neonatal	Hwu et al. [2003]
10		18	c.53delA	p.His18Profs	Frameshift		Neonatal	Tuchman et al. [2002]
11		23	c.67C>T	p.Arg23*	Nonsense		Neonatal	Grompe et al. [1991]
12		26	c.77G>A	p.Arg26Gln	Missense	0%	Neonatal	Grompe et al. [1989]
13		26	c.77G>C	p.Arg26Pro	Missense		Neonatal	Yamaguchi et al. [2006]
14	Intron 1		c.77+1G>T		Splice site error		Female	Tuchman et al. [1997]
15			c.77+1G>A		Splice site error		Female	Yamaguchi et al. [2006]
16			c.77+2dupT		Splice site error		Neonatal	Yamaguchi et al. [2006]
17			c.77+3_6del AAGT		Splice site error		Female	Tuchman et al. [2002]
18			c.77+4A>C		Splice site error	0%	Neonatal	Hoshide et al. [1996]
19			c.77+5G>A		Splice site error		Neonatal	Tuchman et al. [1997]
20			c.78-3C>G		Splice site error	<5%	Female	Bisanzi et al. [2002]
21			c.78-1G>C		Splice site error		Neonatal	Yamaguchi et al. [2006]
22	Exon 2	32	c.94C>T	p.Gln32*	Nonsense		Female	Oppliger Leibundgut et al. [1995]
23		36	c.106C>T	p.Gln36*	Nonsense		Female	Genet et al. [2000]
24		39	c.115G>T	p.Gly39Cys	Missense		Late	Calvas et al. [1998]
25		39	c.116G>A	p.Gly39Asp	Missense		No Information	Shchlechkov et al. [2009]
26		40	c.118C>T	p.Arg40Cys	Missense		Late	Oppliger Leibundgut et al. [1995]
27		40	c.119G>A	p.Arg40His	Missense	6%	Late	Tuchman et al. [1994b]
28		40	c.119G>T	p.Arg40Leu	Missense		Late	Cavicchi et al. [2014
29		41	c.122A>G	p.Asp41Gly	Missense		Female	Yamaguchi et al. [2006]
30		43	c.126_128del TCT	p.Leu43del	In frame indel		Neonatal	Storkanova et al. [2013]
31		43	c.127C>T	p.Leu43Phe	Missense		Female	Oppliger Leibundgut et al. [1997]

32		44	c.131C>T	p.Thr44Ile	Missense		Female	Yoo et al. [1996]
33		45	c.133C>G	p.Leu45Val	Missense		Female	Tuchman et al. [1998]
34		45	c.134T>C	p.Leu45Pro	Missense		Neonatal	Grompe et al. [1989]
35		45	c.135dupA	p.fsX	Frameshift		Female	Yamaguchi et al. [2006]
36		45-47	c.135delA	p.Asn47Thrfs	Frameshift	0%	Neonatal	Calvas et al. [1998]
37		47	c.140_141insG	p.Asn47delinsLysLeufs	Frameshift		Neonatal	Shimadzu et al. [1998]
38		47	c.140A>T	p.Asn47Ile	Missense		Neonatal	Tuchman et al. [1997]
39		47	c.140A>C	p.Asn47Thr	Missense		Female	Yamaguchi et al. [2006]
40		48	c.143T>C	p.Phe48Ser	Missense		Female	Genet et al. [2000]
41		49	c.145A>C	p.Thr49Pro	Missense		Female	Yamaguchi et al. [2006]
42		50	c.148G>A	p.Gly50Arg	Missense		Late	Tuchman et al. [1997]
43		50	c.148G>T	p.Gly50*	Nonsense		Female	Feldmann et al. [1992]
44		52	c.154G>A	p.Glu52Lys	Missense		Neonatal	McCullough et al. [2000]
45		52	c.154G>T	p.Glu52*	Nonsense		Female	McCullough et al. [2000]
46		52	c.155A>G	p.Glu52Gly	Missense		Neonatal	Yamaguchi et al. [2006]
47		52	c.156A>T	p.Glu52Asp	Missense	4%	Late	McCullough et al. [2000]
48		53	c.158T>C	p.Ile53Thr	Missense		Late	Yamaguchi et al. [2006]
49		53	c.158T>G	p.Ile53Ser	Missense		Neonatal	Yamaguchi et al. [2006]
50		55	c.163T>G	p.Tyr55Asp	Missense	28%	Late	Nishiyori et al. [1998]
51		56	c.167T>C	p.Met56Thr	Missense	[54%]	Late	Tuchman et al. [1997]
52		57	c.170T>A	p.Leu57Gln	Missense		Neonatal	Yamaguchi et al. [2006]
53		58	c.174G>A	p.Trp58*	Nonsense		Neonatal	Yamaguchi et al. [2006]
54		59	c.176T>G	Leu59Arg	Missense		Late	Azevedo et al. [2006]
55		60	c.179C>T	p.Ser60Leu	Missense		Female	Tuchman et al. [1997]
56		62	c.184G>C	p.Asp62His	Missense		No Information	Shchlechkov et al. [2009]
57		**62**	**c.185A>G**	**p.Asp62Gly**	Missense		**Female**	**This Report; UCDC Study**
58		63	c.188T>C	p.Leu63Pro	Missense		Female	Oppliger Leibundgut et al. [1997]
59		67	c.200T>G	p.Ile67Arg	Missense		Female	Yamaguchi et al. [2006]
60		69	c.205C>T	p.Gln69*	Nonsense		Female	Climent et al. [1999]
61	Intron 2		c.216+1G>A		Splice site error		Late	Oppliger Leibundgut et al. [1996a]
62			c.216+1G>T		Splice site error	10%	Late	Azevedo et al. [2002]
63			c.217-1G>A		Splice site error		Neonatal	Tuchman et al. [1992]
64	Exon 3	73	c.219T>G	p.Tyr73*	Nonsense		Neonatal	Storkanova et al. [2013]
65		76	c.227T>C	p.Leu76Ser	Missense		Neonatal	Genet et al. [2000]
66		77	c.231G>T	p.Leu77Phe	Missense	[35%]	Late	McCullough et al. [2000]
67		78	c.232C>T	p.Gln78*	Nonsense		Neonatal	Yamaguchi et al. [2006]
68		79	c.236G>A	p.Gly79Glu	Missense	0%	Neonatal	Tuchman et al. [1992]
69		80	c.238A>G	p.Lys80Glu	Missense		Female	Schultz and Salo [2000]
70		80	c.240G>T	p.Lys80Asn	Missense		Late	Galloway et al. [2000]

71		81-82	c.243_245del CTT	p.Leu244del	In frame indel		Female	Tuchman and Plante [1995]
72		82	c.245T>G	p.Leu82*	Nonsense		Female	Tuchman et al. [2002]
73		**82**	**c.245T>A**	**p.Leu82***	Nonsense		**Neonatal**	**This Report; UCDC Study**
74		83	c.248G>A	p.Gly83Asp	Missense		Neonatal	Bartholomew & McClellan [1998]
75		**85**	**c.256dupT**	**p.fsX**	**Frameshift**		**Neonatal**	**This Report; UCDC Study**
76		88	c.264A>T	p.Lys88Asn	Missense	3%	Late	Reish et al. [1993]
77		90	c.268A>G	p.Ser90Gly	Missense	(<20%)	Female	Takanashi et al. [2002]
78		90	c.269G>A	p.Ser90Asn	Missense		Neonatal	McCullough et al. [2000]
79		90	c.270T>G	p.Ser90Arg	Missense		Female	Tuchman et al. [1998]
80		91	c.271delA	p.Thr91Leufs	Frameshift		Female	Genet et al. [2000]
81		92	c.274C>G	p.Arg92Gly	Missense		Female	Yamaguchi et al. [2006]
82		92	c.274C>T	p.Arg92*	Nonsense	0%	Neonatal	Grompe et al. [1991]
83		92	c.275G>T	p.Arg92Leu	Missense		Neonatal	Yamaguchi et al. [2006]
84		92	c.275G>A	p.Arg92Gln	Missense	0%	Neonatal	Grompe et al. [1991]
85		92	c.275G>C	p.Arg92Pro	Missense		Neonatal	Yamaguchi et al. [2006]
86		93	c.277A>G	p.Thr93Ala	Missense		Late	Tuchman and Plante [1995]
87		94	c.281G>C	p.Arg94Thr	Missense		Late	Tuchman et al. [1992]
88		95	c.284T>C	p.Leu95Ser	Missense		Late	McCullough et al. [2000]
89		98	c.292G>A	p.Glu98Lys	Missense	33%	Female	Bisanzi et al. [2002]
90	Intron 3		c.298+1G>A		Splice site error		Neonatal	Garcia-Perez et al. [1995]
91			c.298+1G>T		Splice site error		Late	Yamaguchi et al. [2006]
92			c.298+1_5del GTAAG		Splice site error		Female	Tuchman et al. [1997]
93			c.298+5G>C		Splice site error		Female	Yamaguchi et al. [2006]
94			**c.299-8T>A**		Splice site error		**Late**	**This Report; UCDC Study**
95			**c.299-7A>G**		Splice site error		**Neonatal**	**This Report; UCDC Study**
96	Exon 4	100	c.299G>A	p.Gly100Asp	Missense		Female	Oppliger Leibundgut et al. [1997]
97		100	c.298G>C	Gly100Arg	Missense		Late	Kim et al. [2006]
98		102	c.304G>C	pAla102Pro	Missense		Neonatal	Storkanova et al. [2013]
99		102	c.305C>A	p.Ala102Glu	Missense		Neonatal	Tuchman et al. [1997]
100		105	c.314G>T	p.Gly105Val	Missense		Female	Yamaguchi et al. [2006]
101		105	c.314G>A	p.Gly105Glu	Missense		Late	Cavicchi et al. [2014]
102		106	c.316G>A	p.Gly106Arg	Missense		Female	McCullough et al. [2000]
103		106	c.317G>A	p.Gly106Glu	Missense	<20%	Female	Takanashi et al. [2002]
104		106	c.317G>T	p.Gly106Val	Missense		Female	Yamaguchi et al. [2006]
105		**109**	**c.327T>C**	**p.Cys109Arg**	Missense		**Female**	**This Report; UCDC Study**
106		109-110	c.327delT	p.Cys109Cysfsx	Frameshift		Neonatal	Calvas et al. [1998]
107			c.341-342delAA	p.fsX	Frameshift		Female	Azevedo et al. [2006]
108		111	c.332T>C	p.Leu111Pro	Missense		No Information	Grompe et al. [1989]

109		117	c.350A>G	p.His117Arg	Missense	18%	Late	Matsuura and Matsuda [1998]
110		117	c.350A>T	p.His117Leu	Missense		Late	Tuchman et al. [1994a]
111		120	c.358_359delGT	p.Val120Glufs	Frameshift		Female	Yamaguchi et al. [2006]
112		122	c.364_365insTT	p.Glu122delinsValLysfs	Frameshift		Female	Yamaguchi et al. [2006]
113		125	c.374C>T	p.Thr125Met	Missense	<1%	Neonatal	Gilbert-Dussardier et al. [1996]
114		125	c.375delG	p.Thr125Thrfs	Frameshift		Female	Yamaguchi et al. [2006]
115		126	c.377A>G	p.Asp126Gly	Missense		Neonatal	Matsuura et al. [1994]
116		129	c.386G>C	p.Arg129Pro	Splice site error		Female	Yamaguchi et al. [2006]
117		129	c.386G>A	p.Arg129His	Splice site error	3.50%	Late	Tuchman et al. [1994b]
118		129	c.386G>T	p.Arg129Leu	Splice site error		Late	Strautnieks and Malcolm [1993]
119		129	c.385C>T	p.Arg129Cys	Splice site error		No Information	Shchlechkov et al. [2009]
120	Intron 4		c.386+1G>T		Splice site error		Late	Yamaguchi et al. [2006]
121			c.386+1G>A		Splice site error		Neonatal	Yamaguchi et al. [2006]
122			c.386+1G>C		Splice site error		Female	Ogino et al. [2007]
123			c.386+2T>C		Splice site error		Neonatal	Yamaguchi et al. [2006]
124			**c.386+5G>A**		Splice site error		**Neonatal**	**This Report; UCDC Study**
125			c.387-2A>T		Splice site error		Neonatal	Carstens et al. [1991]
126			c.387-2A>C		Splice site error		Neonatal	McCullough et al. [2000]
127			c.387-2A>G		Splice site error		Neonatal	McCullough et al. [2000]
128	Exon 5	130 - 131	c.390_391insTTA	p.Val130_Leu131insLeu	In frame indel		Female	Tuchman et al. [2002]
129		131	c.392T>C	p.Leu131Ser	Missense		Late	Yamaguchi et al. [2006]
130		132	c.394T>C	p.Ser132Pro	Missense		Late	Bisanzi et al. [2002]
131		132	c.395C>T	p.Ser132Phe	Missense	[78.6%]	Late	Gyato et al. [2004]
132		135	c.403delG	p.Ala135Glnfs	Frameshift		Neonatal	Tuchman et al. [1992]
133		135	c.404C>A	p.Ala135Glu	Missense		Late	Yamaguchi et al. [2006]
134		136	c.407A>T	p.Asp136Val	Missense		Late	Yamaguchi et al. [2006]
135		137	c.409G>C	p.Ala137Pro	Missense		Female	Azevedo et al. [2006)]
136		137	c.409G>A	p.Ala137Thr	Missense		Female	Yamaguchi et al. [2006]
137		139	c.416T>C	p.Leu139Ser	Missense		Female	Tuchman et al. [1997]
138		140	c.418G>C	p.Ala140Pro	Missense		Neonatal	Yamaguchi et al. [2006]
139		140	c.419C>A	pAla140Asp	Missense		No Information	Shchlechkov et al. [2009]
140		141	c.421C>G	p.Arg141Gly	Missense		Female	Yamaguchi et al. [2006]
141		141	c.421C>T	p.Arg141*	Nonsense		Neonatal	Hata et al. [1989]
142		141	c.422G>A	p.Arg141Gln	Missense	0%	Neonatal	Maddalena et al. [1988b]
143		141	c.422G>C	p.Arg141Pro	Missense		Female	Tuchman et al. [1997]
144		142	c.425T>A	p.Val142Glu	Missense		Late	Tuchman et al. [2002]
145		143	c.429T>A	p.Tyr143*	Nonsense		No Information	Makhtar et al. [2013]
146		144	c.430A>T	p.Lys144*	Nonsense		Female	Tuchman and Plante [1995]

147		146	c.437C>G	p.Ser146*	Nonsense		Neonatal	Genet et al. [2000]
148		148	c.443T>C	p.Leu148Ser	Missense		Neonatal	Yamaguchi et al. [2006]
149		148	c.443T>G	p.Leu148Trp	Missense		Female	McCullough et al. [2000]
150		148	c.444G>C	p.Leu148Phe	Missense		Female	Komaki et al. [1997]
151		148	c.444G>T	p.Leu148Phe	Missense	17%	Female	Matsuura and Matsuda [1998]
152		150-151	c.449delC	p.fsX	Frameshift		Female	Tuchman et al. [2002]
153		151	c.452T>G	p.Leu151Arg	Missense		Female	Yamaguchi et al. [2006]
154		152	c.455C>T	p.Ala152Val	Missense	3.70%	Late	Kogo et al. [1998]
155		154	c.460G>T	p.Glu154*	Nonsense	0%	Neonatal	Grompe et al. [1989]
156		154	c.461_471del	p.Glu154Alafs*18	Frameshift		Neonatal	Storkanova et al. [2013]
157		155	c.463G>T	p.Ala155Ser	Missense		Female	Tuchman et al. [2002]
158		155	c.463G>C	p.Ala155Pro	Missense		Female	Yamaguchi et al. [2006]
159		155	c.464C>A	p.Ala155Glu	Missense		Neonatal	Yamaguchi et al. [2006]
160		158	c.472C>T	p.Pro158Ser	Missense		Late	Storkanova et al. [2013]
161		159	c.476T>C	p.Ile159Thr	Missense	1.50%	Late	Garcia-Perez et al. [1995]
162		159	c.477T>G	p.Ile159Met	Missense		Late	Ben-Ari et al. [2010]
163		160	c.479T>G	p.Ile160Ser	Missense		Female	Climent and Rubio [2002b]
164		160	c.479T>A	p.Ile160Asn	Missense		Neonatal	Yamaguchi et al. [2006]
165		160	c.479T>C	p.Ile160Thr	Missense		Neonatal	Yamaguchi et al. [2006]
166		161	c.481A>G	p.Asn161Asp	Missense		Neonatal	Genet et al. [2000]
167		161	c.482A>G	p.Asn161Ser	Missense		Neonatal	Tuchman and Plante [1995]
168		161	c.483T>A	p.Asn161Lys	Missense	<10%	Female	Takanashi et al. [2002]
169		161	c.483T>G	p.Asn161Lys	Missense	<10%	Female	Takanashi et al. [2002]
170		162	c.484G>C	p.Gly162Arg	Missense		Female	Yamaguchi et al. [2006]
171		162	c.484G>A	p.Gly162Arg	Missense		Neonatal	Feldmann et al. [1992]
172		162	c.485G>A	p.Gly162Glu	Missense		Neonatal	Yamaguchi et al. [2006]
173		164	c.490T>C	p.Ser164Pro	Missense		Neonatal	Yamaguchi et al. [2006]
174		164	c.491C>G	p.Ser164*	Nonsense		Female	Matsuda and Tanase [1997]
175		165	c.493G>T	p.Asp165Tyr	Missense		Late	Genet et al. [2000]
176		167	c.501C>A	p.Tyr167*	Nonsense	0%	Neonatal	Garcia-Perez et al. [1995]
177		167	c.501C>G	p.Tyr167*	Nonsense		Neonatal	Shimadzu et al. [1998]
178		168	c.503A>C	p.His168Pro	Missense		Late	Yamaguchi et al. [2006]
179		168	c.503A>G	p.His168Arg	Missense		Female	Vella et al. [1996]
180		168	c.504T>A	p.His168Gln	Missense	[69%]	Late	Tuchman et al. [1997]
181		169	c.505C>G	p.Pro169Ala	Missense		Female	Tuchman et al. [2002]
182		169	c.506C>T	p.Pro169Leu	Missense		Neonatal	Genet et al. [2000]
183		**169**	**c.506C>A**	**p.Pro169His**	Missense		**Female**	**This Report; UCDC Study**
184		172	c.514A>T	p.Ile172Phe	Missense		Female	Climent et al. [1999]
185		172	c.516C>G	p.Ile172Met	Missense		Neonatal	Matsuura et al. [1994]
186		172	c.515T>A	Ile172Asn	Missense		Late	Ogino et al. [2007]
187		174	c.520G>C	p.Ala174Pro	Missense		Female	Tsai et al. [1993]

188		175	c.524A>G	p.Asp175Gly	Missense		Late	Genet et al. [2000]
189		175	c.524A>T	p.Asp175Val	Missense		Female	Tuchman et al. [1997]
190		176	c.526T>C	p.Tyr176His	Missense		Neonatal	Tuchman et al. [2002]
191		176	c.527A>G	p.Tyr176Cys	Missense	19%	Late	Oppliger Leibundgut et al. [1996b]
192		176	c.527A>C	p.Tyr176Leu	Missense		Female	Azevedo et al. [2006]
193		178	c.533C>T	p.Thr178Met	Missense		Neonatal	Oppliger Leibundgut et al. [1995]
194		177-178	c.530_533 dupTCAC	p.fsX	Frameshift		Neonatal	Gilbert-Dussadier et al. [1996]
195		178-179	c.532_537del ACGCTC	p.Thr178_Leu179del	In frame indel		Neonatal	Shimadzu et al. [1998]
196		179	c.535C>T	p.Leu179Phe	Missense		Late	Fantur et al. [2013]
197		179	c.536T>C	p.Leu179Pro	Missense		Neonatal	Yamaguchi et al. [2006]
198		**180**	**c.538C>T**	**p.Gln180***	Nonsense		**Neonatal**	**This Report; UCDC Study**
199		180	c.[539A>C (+) 540G>C]	p.Gln180Pro	Missense	<10%	Neonatal	Hübler et al. [2001]
200		180	c.540G>C	p.Gln180His	Splice site error	7.1% [43%]	Neonatal	Tuchman et al. [1997]
201	Intron 5		c.540+1G>C		Splice site error		Neonatal	Oppliger Leibundgut et al. [1996a]
202			c.540+2T>C		Splice site error	0%	Neonatal	Matsuura et al. [1995]
203			c.540+2T>A		Splice site error		Neonatal	Yamaguchi et al. [2006]
204			c.541-2A>G		Splice site error		Neonatal	Genet et al. [2000]
205			c.540+265 G>A		Splice site error		Late	Ogino et al. [2007]
206			c540+2T>G		Splice site error		No Information	Shchlechkov et al.]2009]
207	Exon 6	181	c.542A>G	p.Glu181Gly	Missense		Neonatal	Tuchman et al. [1998]
208		182	c.545A>T	p.His182Leu	Missense		Neonatal	Tuchman et al. [1994a]
209		183	c.547T>G	p.Tyr183Asp	Missense		Female	Oppliger Leibundgut et al. [1997]
210		183	c.548A>G	p.Tyr183Cys	Missense		Neonatal	Reish et al. [1993]
211		186	c.557T>C	p.Leu186Pro	Missense		Female	Azevedo et al. [2006]
212		**188**	**c.561delA**	**p.Gln188fs**	Frameshift		**Female**	**This Report; UCDC Study**
213		188	c.562G>C	p.Gly188Arg	Missense	2%	Neonatal	Gilbert-Dussadier et al. [1996]
214		188	c.562_563del GG	p.Gly188SfsX36	Frameshift		No Information	Shchlechkov et al. [2009]
215		188	c.563G>T	p.Gly188Val	Missense		Female	Climent et al. [1999]
216		188	c.563G>C	p.Gly188Ala	Missense		No Information	Shchlechkov et al. [2009]
217		190	c.568delA	p.T190PfsX16	Frameshift		No Information	Shchlechkov et al. [2009]
218		191	c.571C>T	p.Leu191Phe	Missense	5.70%	Late	Climent and Rubio [2002b]
219		191	c.571delC	p.Leu191SerfsX15	Frameshift		Neonatal	Kim et al. [2006]
220		191	c.572T>G	p.Leu191Arg	Missense		Neonatal	Yamaguchi et al. [2006]
221		192	c.576C>G	p.Ser192Arg	Missense		Neonatal	Matsuura et al. [1993]
222		193	c.577T>C	p.Trp193Arg	Missense		Female	Yamaguchi et al. [2006]
223		193	c.577T>G	p.Trp193Gly	Missense		Female	Yamaguchi et al. [2006]

224		193	c.578G>A	p.Trp193*	Nonsense		Neonatal	Shimadzu et al. [1998]
225		193	c.579G>C	p.Trp193Cys	Missense		No Information	Shchlechkov et al. [2009]
226		**194**	**c.581T>C**	**p.Ile194Thr**	Missense		**Late**	**This Report; UCDC Study**
227		195	c.583G>A	p.Gly195Arg	Missense	0%	Neonatal	Tuchman et al. [1994b]
228		195	c.583delG	p.Asp196Metfs	Frameshift		Female	Climent and Rubio [2002b]
229		196	c.586G>A	p.Asp196Asn	Missense		Late	Yamaguchi et al. [2006]
230		196	c.586G>T	p.Asp196Tyr	Missense		Neonatal	Tuchman et al. [1998]
231		196	c.586G>C	p.Asp196His	Missense		No Information	Lin et al. [2010]
232		196	c.587A>T	p.Asp196Val	Missense	7%	Neonatal	Matsuura et al. [1993]
233		197	c.589G>A	p.Gly197Arg	Missense		Female	Climent et al. [1999]
234		197	c.590G>A	p.Gly197Glu	Missense		Female	Tuchman et al. [1998]
235		197	c.589G>T	p.Gly197Trp	Missense		No Information	Shchlechkov et al. [2009]
236		198	c.593A>T	p.Asn198Ile	Missense		Neonatal	Yamaguchi et al. [2006]
237		198	c.594C>A	p.Asn198Lys	Missense		Female	Popowska et al. [1999]
238		199	c.595A>G	p.Asn199Asp	Missense		Female	Yamaguchi et al. [2006]
239		199	c.596A>G	p.Asn199Ser	Missense		Neonatal	Tuchman et al. [2002]
240		199-200	c.(597_598) delTA	p.fsX	Frameshift		Neonatal	Tuchman et al. [1994b]
241		201	c.602T>C	p.Leu201Pro	Missense		Neonatal	Shimadzu et al. [1998]
242		202	c.604C>T	p.His202Tyr	Missense	[49%]	Late	Tuchman et al. [1997]
243		202	c.605A>C	p.His202Pro	Missense		Female	Staudt et al. [1998]
244		203	c.608C>G	p.Ser203Cys	Missense		Female	Tuchman et al. [1994a]
245		205	c.613A>G	p.Met205Val	Missense		Neonatal	Genet et al. [2000]
246		205	c.614T>C	p.Met205Thr	Missense		Neonatal	Kim et al. [2006]
247		206	c.617T>G	p.Met206Arg	Missense		Neonatal	Tuchman et al. [1997]
248		206	c.618G>C	p.Met206Ile	Missense		Female	Climent and Rubio [2002b]
249		207	c.620G>A	p.Ser207Asn	Missense		Neonatal	Yamaguchi et al. [2006]
250		207	c.621C>A	p.Ser207Arg	Missense		Neonatal	Shimadzu et al. [1998]
251		208	c.622G>A	p.Ala208Thr	Missense	4%	Late	van Diggelen et al. [1996]
252		209	c.626C>T	p.Ala209Val	Missense	1% [1.4%]	Neonatal	Garcia-Perez et al. [1995]
253		210	c.628A>C	p.Lys210Glu	Missense		Female	Storkanova et al. [2013]
254		210	c.630A>C	pLys210Asn	Missense		Female	Azevedo et al. [2006]
255		210	c.628A>C	p.Lys210Gln	Missense	0%	Female	Valik et al. [2004]
256		213	c.637T>A	p.Met213Lys	Missense		Female	Oppliger Leibundgut et al. [1997]
257		**213**	**c.637T>C**	**p.Met213Thr**	Missense		**Female**	**This Report; UCDC Study**
258		**213**	**c.637T>G**	**p.Met213Arg**	Missense		**No Information**	**This Report**
259		214	c.640C>T	p.His214Tyr	Missense		Neonatal	Yoo et al. [1996]
260		215	c.643C>T	p.Leu215Phe	Missense	17%	Female	Ueta et al. [2001]
261		215-216	c.645_646ins T	p.Gln216delinsSerGly fs	Frameshift		Neonatal	Tuchman et al. [1994a]
262		216	c.646C>G	p.Gln216Glu	Missense		Neonatal	Grompe et al. [1989]

263		217	c.650C>A	p.Ala217Glu	Missense		Female	Yamaguchi et al. [2006]
264		218	c.653C>T	p.Ala218Val	Missense		No Information	Shchlechkov et al. [2009]
265		220	c.658C>G	p.Pro220Ala	Missense	35%	Late	Oppliger Leibundgut et al. [1996b]
266		220	c.659C>T	p.Pro220Leu	Missense		Neonatal	Yamaguchi et al. [2006]
267		221	c.663G>C	p.Lys221Asn	Missense		Neonatal	Yamaguchi et al. [2006]
268		221	c.663G>A	p.Lys221Lys	Splice site error	8%	Late	Shimadzu et al. [1998]
269	Intron 6		c.663+1G>A		Splice site error		Female	Tuchman et al. [1997]
270			c.663+1G>T		Splice site error		Female	Oppliger Leibundgut et al. [1996a]
271			c.663+1delG		Splice site error		No Information	Shchlechkov et al. [2009]
272			c.663+2T>C		Splice site error		Neonatal	Tuchman et al. [1997]
273			c.663+2dupT		Splice site error		Female	Tuchman and Plante [1995]
274			c.664-1G>A		Splice site error		Neonatal	Tuchman et al. [2002]
275			c.664-1delG	p.fsX	Frameshift		Female	Tuchman et al. [1997]
276	Exon 7	222	c.664-667delinsAC	p.Gly222Thrfs*2	Frameshift		Neonatal	Lee et al. [2014]
277		224	c.670G>T	p.Glu224*	Nonsense		No Information	Shchlechkov et al. [2009]
278		225	c.673C>A	p.Pro225Thr	Missense	[42%]	Late	Tuchman et al. [1994b]
279		225	c.674C>G	p.Pro225Arg	Missense	0%	Neonatal	Garcia-Perez et al. [1997]
280		225	c.674C>T	p.Pro225Leu	Missense	0% [0.45%]	Neonatal	Hentzen et al. [1991]
281		233	c.698C>T	p.Ala233Val	Missense		Neonatal	Yamaguchi et al. [2006]
282		234	c.700G>T	p.Glu234*	Nonsense		Neonatal	Yamaguchi et al. [2006]
283		239	c.716A>T	p.Glu239Val	Missense		Neonatal	Yamaguchi et al. [2006]
284		239	c.716A>G	p.Glu239Gly	Missense		Late	Yamaguchi et al. [2006]
285		239	c.717G>C	p.Glu239Asp	Missense		Female	Yamaguchi et al. [2006]
286		239	c.717G>A	p.Glu239Glu	Splice site error		Female	Tuchman et al. [1997]
287	Intron 7		c.717+1G>T		Splice site error		Female	Tuchman et al. [2002]
288			c.717+1G>A		Splice site error		Neonatal	Genet et al. [2000]
289			c.717+2T>C		Splice site error		Neonatal	Carstens et al. [1991]
290			c.717+3A>G		Splice site error		Neonatal	Carstens et al. [1991]
291			c.717+7_22delTCTTTACATGTAAAGC		Splice site error	0-1.5%	Neonatal	Calvas et al. [1998]
292			c.718-2A>G		Splice site error		Female	Popowska et al. [1999]
293	Exon 8		c.718-4_729delCTAGAATGGTACCAAG		Splice site error		Female	Yamaguchi et al. [2006]
294		242	c.725C>T	p.Thr242Ile	Missense		Late	Tuchman et al. [1997]
295		244	c.731T>A	p.Leu244Gln	Missense	8%	Late	Calvas et al. [1998]

296		247	c.740C>A	p.Thr247Lys	Missense	0%	Neonatal	Tuchman and Plante [1995]
297		244-247	c.731_739delTGTTGCTGA		In frame indel		Female	Calvas et al. [1998]
298		249	c.746A>G	p.Asp249Gly	Missense		Neonatal	Kim et al. (2006)
299		**250**	**c.749C>T**	**p.Pro250Leu**	**Missense**		**Late**	**This Report**
300		253	c.757G>A	p.Ala253Thr	Missense		Neonatal	Yamaguchi et al. [2006]
301		253	c.757G>C	p.Ala253Pro	Missense		Neonatal	Yamaguchi et al. [2006]
302		253	c.759delA	p.fsX	Frameshift		Neonatal	Yamaguchi et al. [2006]
303		**254**	**c.760A>T**	**p.Ala254***	Nonsense		**Female**	**This Report; UCDC Study**
304		255	c.764A>C	p.His255Pro	Missense		Female	Tuchman et al. [1998]
305		260	c.779T>C	p.Leu260Ser	Missense		Female	Yamaguchi et al. [2006]
306		**262**	**c.784_792dup9**	**p.thr262_Thr264dupTDT**	In frame indel		**Neonatal**	**This Report; UCDC Study**
307		262	c.785C>A	p.Thr262Lys	Missense	26%	Late	Giorgi et al. [2000]
308		262	c.785C>T	p.Thr262Ile	Missense		Late	Yamaguchi et al. [2006]
309		263	c.787G>A	p.Asp263Asn	Missense		Female	Tuchman et al. [1997]
310		263	c.788A>G	p.Asp263Gly	Missense		Female	Tuchman et al. [1998]
311		264	c.790A>G	p.Thr264Ala	Missense	22%	Late	Matsuura et al. [1993]
312		264	c.791C>A	p.Thr264Asn	Missense		No Information	Hwu et al. [2003]
313		264	c.791C>T	p.Thr264Ile	Missense		Late	Shimadzu et al. [1998]
314		265	c.793T>C	p.Trp265Arg	Missense		Late	Yamaguchi et al. [2006]
315		265	c.794G>T	p.Trp265Leu	Missense	56%	Late	Giorgi et al. [2000]
316		265	c.795G>A	p.Trp265*	Nonsense		Neonatal	Yamaguchi et al. [2006]
317		265-268	c.796_805del	p.Ile265_Gly268delinsAspfsX19	Frameshift		Neonatal	Kim et al. [2006]
318		267	c.799A>C	p.Ser267Arg	Missense		Female	Shimadzu et al. [1998]
319		268	c.803T>C	p.Met268Thr	Missense	6.70%	Late	Matsuura et al. [1993]
320		268	c.802A>G	p.Met268Val	Missense		No Information	Jamroz at al. [2013]
321		269	c.806G>A	p.Gly269Glu	Missense	2%	Neonatal	Zimmer et al. [1995]
322		270	c.808C>T	p.Gln270*	Nonsense		Neonatal	McCullough et al. [2000]
323		270	c.809A>C	p.Gln270Pro	Missense		Female	Yamaguchi et al. [2006]
324		271-272	c.810_811delAGinsC	p.fsX	Frameshift		Neonatal	Khoo et al. [1999]
325		272-273	c.817_819delGAG	p.Glu273del	In frame indel	5%	Late	Segues et al. [1996]
326		273	c.818delA	p.Glu273Glyfs	Frameshift		Neonatal	Yamaguchi et al. [2006]
327		277	c.829C>T	p.Arg277Trp	Missense	5% [59%]	Late	Finkelstein et al. [1990a]
328		277	c.830G>A	p.Arg277Gln	Missense	7%	Late	Tuchman et al. [1994b]
329		277	c.830G>T	p.Arg277Leu	Missense		Late	Tuchman et al. [2002]
330		279	c.835C>T	p.Gln279*	Nonsense		Neonatal	Tuchman et al. [2002]
331		281	c.842T>C	p.Phe281Ser	Missense		Neonatal	Kim et al. [2006]

332		284	c.853delC	p.Phe284fsX38	Frameshift		Neonatal	Kim et al. [2006]
333		285	c.853C>T	p.Gln285*	Nonsense		Female	Storkanova et al. [2013]
334		**287**	**c.860A>C**	**p.Thr287Pro**	Missense		**Female**	**This Report**
335		**287**	**c.861insAC**	**p.fsX**	Frameshift		**Female**	**This Report; UCDC Study**
336		286-289	c.867G>A r.856_867delGTTACAATGAG	p.Val286_Lys289del	In frame indel		Late	Storkanova et al. [2013]
337		**289**	**c.867G>C**	**p.Lys289Asp**	Missense		**Neonatal**	**This Report; UCDC Study**
338		289	c.867G>T	p.Lys289Asn	Missense	0%	Neonatal	Tuchman et al. [2002]
339	Intron 8		c.867+1G>A		Splice site error		Neonatal	Tuchman et al. [1998]
340			c.867+1G>T		Splice site error		Neonatal	Oppliger Leibundgut et al. [1996a]
341			c.867+1126A>G		Splice site error		Late	Engel et al. [2008]
342			c.868-3T>C		Splice site error		Late	Lee et al. [2014]
343	Exon 9	292	c.875delA	p.Val293Leufs	Frameshift		Neonatal	Yamaguchi et al. [2006]
344		294	c.882delT	p.Ala295Profs	Frameshift		Neonatal	Reish et al. [1993]
345		297	c.889G>T	p.Aso297Tyr	Missense		No Information	Shchlechkov et al. [2009]
346		297-298	c.889_892delGACT	p.fsX	Frameshift	0%	Neonatal	Yamanouchi et al. [2002]
347		298	c.892_893delTG	p.Trp298Aspfs	Frameshift		Female	Schimanski et al. [1996]
348		**298**	**c.892T>C**	**p.Trip298Arg**	Missense		**Female**	**This Report; UCDC Study**
349		298	c.893G>C	p.Trp298Ser	Missense	0%	Neonatal	Ensenauer et al. [2005]
350		**301**	**c.902T>C**	**p.Leu301Ser**	Missense		**Female**	**This Report; UCDC Study**
351		301	c.903A>T	p.Leu301Phe	Missense	3%	Late	Climent and Rubio [2002b]
352		302	c.904C>T	p.His302Tyr	Missense	0%	Neonatal	Oppliger Leibundgut et al. [1996b]
353		302	c.905A>G	p.His302Arg	Missense		Neonatal	Genet et al. [2000]
354		302	c.905A>T	p.His302Leu	Missense		Female	Gilbert-Dussadier et al. [1996]
355		302	c.906C>G	p.His302Gln	Missense		Late	Tuchman et al. [1997]
356		302	c.906delC	p.Cys303Alafs	Frameshift		Female	Yamaguchi et al. [2006]
357		303	c.907T>C	p.Cys303Arg	Missense		Neonatal	Calvas et al. [1998]
358		303	c.907T>G	p.Cys303Gly	Missense		Neonatal	Tuchman et al. [2002]
359		303	c.908G>A	p.Cys303Tyr	Missense		Female	Tuchman et al. [1997]
360		304	c.912G>T	p.Leu304Phe	Missense	6% [74%]	Late	Tuchman et al. [1992]
361		305	c.914C>G	p.Pro305Arg	Missense		Neonatal	Yamaguchi et al. [2006]
362		305	c.914C>A	p.Pro305His	Missense		Female	Climent and Rubio [2002b]
363		306	c.916A>T	p.Arg306*	Nonsense		No Information	Shchlechkov et al. [2009]
364		306	c.917G>C	p.Arg306Thr	Missense		No Information	Meng et al. [2013]
365		309-310	c.(925-927)delGAA	p.Glu309del	In frame indel		Late	Tuchman et al. [1994b]

366		310	c.928G>T	p.Glu310*	Nonsense		Neonatal	Reish et al. [1993]
367		310	c.929_931del AAG	p.Glu310del	In frame indel		Late	Storkanova et al. [2013]
368		310	c.929A>G	p.Glu310Gly	Missense		Late	Yamaguchi et al. [2006]
369		311	c.931G>A	p.Val311Met	Missense		Late	Yamaguchi et al. [2006]
370		**311**	**c.932T>A**	**p.Val311Glu**	Missense		**Neonatal**	**This Report; UCDC Study**
371		314	c.940_942del GAA	p.Glu314del	In frame indel		Female	Yamaguchi et al. [2006]
372		315	c.943G>T	p.Val315Phe	Missense		Female	Yamaguchi et al. [2006]
373		315	c.944T>A	p.Val315Asp	Missense		Female	Tuchman et al. [2002]
374		315	c.944T>G	p.Val315Gly	Missense		Female	Tuchman et al. [2002]
375		316	c.947T>C	p.Phe316Ser	Missense		Female	Tuchman et al. [2002]
376		318	c.953C>T	p.Ser318Phe	Missense		Female	Genet et al. [2000]
377		320	c.958C>T	p.Arg320*	Nonsense	10-15%	Neonatal	Yoo et al. [1996]
378		320	c.959G>T	p.Arg320Leu	Missense	[3.9%]	Neonatal	Grompe et al. [1991]
379		321	c.962C>A	p.Ser321*	Nonsense		Neonatal	Tuchman et al. [2002]
380		**322**	**c.964C>G**	**p.Leu322Val**	Missense		**Female**	**This Report; UCDC Study**
381		322	c.965T>C	p.Leu322Pro	Missense		No Information	Shchlechkov et al.[(2009]
382		323	c.967G>A	p.Val323Met	Missense		Late	Kim et al. [2006]
383		326	c.976G>A	p.Glu326Lys	Missense		Female	Popowska et al. [1999]
384		328	c.982G>T	p.Glu328*	Nonsense		Neonatal	Yamaguchi et al. [2006]
385		330	c.988A>T; 989_990delGA	p.fsX	Frameshift		Female	Climent and Rubio [2002b]
386		330	c.988A>G	p.Arg330Gly	Missense		Female	Tuchman et al. [1997]
387		331	c.991A>T	p.Lys331*	Nonsense		Female	Yamaguchi et al. [2006]
388		332	c.994T>A	p.Trp332Arg	Missense	0%	Neonatal	Rapp et al. [2001]
389		332	c.995G>A	p.Trp332*	Nonsense		Neonatal	Yamaguchi et al. [2006]
390		332	c.996G>A	p.Trp332*	Nonsense		Neonatal	Matsuura et al. [1994]
391		332	c.995G>C	p.Trp332Ser	Missense		Neonatal	Wang et al. [2014]
392		335	c.1005G>A	p.Met335Ile	Missense		Neonatal	Tuchman et al. [2002]
393	Intron 9		c.1005+1G>T		Splice site error		Neonatal	Tuchman et al. [1997]
394			c.1005+2T>C		Splice site error		Neonatal	Tuchman et al. [2002]
395			c.1006-3C>G		Splice site error	2.70%	Late	Climent and Rubio [2002b]
396			c.1006-1G>A		Splice site error	[23%]	Neonatal	Gyato et al. [2004]
397			c.1005_1091 C>G		Splice site error		Late	Engel et al. [2008]
398	Exon 10	336	c.1006G>T	p.Ala336Ser	Missense		Late	Tuchman et al. [1998]
399		337	c.1009G>C	p.Val337Leu	Missense	<5%	Late	Matsuda and Tanase [1997]
400		339	c.1015G>C	p.Val339Leu	Missense		Neonatal	Tuchman et al. [1997]

401		339	c.1016T>G	p.Val339Gly	Missense		Neonatal	Wang et al. [2014]
402		340	c.1018T>C	p.Ser340Pro	Missense		Female	Oppliger Leibundgut et al. [1997]
403		341	c.1022T>C	p.Leu341Pro	Missense		Female	Climent and Rubio [2002b]
404		**343**	**c.1028C>G**	**p.Thr343Arg**	Missense		**Neonatal**	**This Report**
405		343	c.1028C>A	p.Thr343Lys	Missense		Female	Tuchman and Plante [1995]
406		345	c.1033T>C	p.Tyr345His	Missense		Late	Yamaguchi et al. [2006]
407		345	c.1033T>G	p.Tyr345Asp	Missense		Female	Tuchman et al. [1992]
408		**345**	**c.1034A>G**	**p.Tyr345Cys**	Missense		**Female**	**This Report; UCDC Study**
409		347	c.1039C>A	p.Pro347Thr	Missense		Female	Yamaguchi et al. [2006]
410		**347**	**c.1039C>T**	**p.Pro347Ser**	Missense		**Neonatal**	**This Report; UCDC Study**
411		**347**	**c.1040C>T**	**p.Pro347Leu**	Missense		**Female**	**This Report**
412		348	c.1042C>T	p.Gln348*	Nonsense		Female	Oppliger Leibundgut et al. [1997]
413		348	c.1043delA	p.Gln348Argfs*47	Frameshift		Female	Storkanova et al. [2013]
414		**348**	**c.1046T>C**	**p.Leu349Pro**	Missense		**Female**	**This Report; UCDC Study**
415		354	c.1061T>G	p.Phe354Cys	Missense	1.80%	Late	Myers and Shook [1996]
416		**355**	**c.1063T>C**	**p.*355Glu**	**Extending**		**Female**	**This Report; UCDC Study**
417		355	c.1065A>T	p.*355Cysext*14	Extending		Late	Storkanova et al. [2013]

[a]Nucleotide +1 is the A of the translation initiation codon of the NM_000531.3.

[b]For deletions or insertions, the cDNA nucleotide number is given starting with the A of the translation initiation codon.

[c](%) residual activity in liver or intestine or determined by expression studies; [^{15}N] residual nitrogen incorporation into urea.

[d]Neonatal – hyperammonemia within the first week of life, severe phenotype; Late – late onset, milder phenotype.

[e]First report of the mutation. Bold indicate mutations first reported here and mutations obtained from the UCDC study.

Last PubMed database search was carried out on August 29, 2014.

Table s2.docx

Table S2. Large duplications, deletions and rearrangements associated with OTC deficiency.

Duplications

No.	Size	Dulicated Region	HGVS description	Disease onset	Reference[b]
1	approx. 0.63 Mb	TSPAN7-OTC-RGPR-SRPX	–[a]	Asymptomatic Male	Shchelochkov et al. [2009]

Complex Rearrangements

No.		Description	HGVS description	Disease Onset	Reference
1	4.5 kb and 5.5 kb	Exon 1-4 duplication; exon 5 deletion; 3'end deletion	–	Female	Shchelochkov et al. [2009]
2	250 kb and 8.83 Mb	205 kb deletion within IL1RAPL1; GK through OTC deletion	Xp11.4p21.2	Female	Shchelochkov et al. [2009]

Deletions

No.	Size	Deleted region	HGVS description	Disease Onset	Reference
1	≥ 10 Mb	X short arm	46,Xdel(X)(p11)	Female	Joost et al [2010]
2		NROB1 through OTC	–	Female	Francke U [1984]
3		NROB1 through OTC	–	Neonatal	Old et al. [1985]
4		DMD through OTC	–	Neonatal	Segues et al. [1995]
5		DMD through OTC	–	Female	Climent et al. [1999]
6		MAGEB1 through OTC	–	Female	Shchelochkov et al. [2009]
7	~5-10 Mb	DMD intron44 through OTC	–	Female	Jakubiczka et al [2007]
8		FTHL17 through OTC	–	Female	Balasubramaniam et al [2010]
9		GK through OTC	–	Female	Shchelochkov et al. [2009]
10		DMD through OTC	–	Female	Shchelochkov et al. [2009]
11		DMD through OTC exon 4	Xp11.4-21.2	Female	Wong et al. [2008]
12	<5 Mb	RPGR through OTC	–	Female	Segues et al [1995]
13		TMEM through OTC	Xdel(p11.4)(21-1)	Neonatal	Deardorff et al [2008]
14		RPGR through TSPAN7	–	Neonatal	Arranz et al. [2007]
15		RPGR through TSPAN7	1,023,226 bp to 2,077,444 bp relative to NT_079573	Neonatal	Ono et al. [2010]
16		TMEM4 through OTC	–	Neonatal	Shchelochkov et al. [2009]
17		SRPX through OTC	–	Female	Shchelochkov et al. [2009]
18		TSPAN7 through OTC	–	Neonatal	Quental et al [2009]
19	Whole OTC gene	Exon 1-10	–	Female	Wong et al. [2008]
20		Exon 1-10	–	Neonatal	Grompe et al [1991]
21		Exon 1-10	–	Neonatal	Grompe et al [1991]
22		Exon 1-10	–	Neonatal	Grompe et al [1991]
23		Exon 1-10	–	Neonatal	Rozen et al [1986]
24		Exon 1-10	–	Neonatal	Tuchman et al [2002]
25		Exon 1-10	–	Neonatal	Suess et al. [1992]
26		Exon 1-10	–	Female	Shchelochkov et al. [2009]
27		Exon 1-10	–	Female	Shchelochkov et al. [2009]

28		Exon 1-10	–	Neonatal	Yamaguchi et al [2006]
No.	**Size**	**Deleted region**	**HGVS description**	**Disease Onset**	**Reference**
29		Exon 1-10	g.1276_445398delins63	Neonatal	Storkanova et al. [2013]
30	119 kb	RPGR3 through OTC exon 1	arr Xp11.4(37982502-38102292)x1	Female and Neonatal	Quintero-Rivera et al. [2010]
31		Exon 1	–	Female	Lee et al. [2014]
32		Exons 1-3	–	Neonatal	Tuchman et al. [1997]
33	>24 kb	Exons 1-3	–	Female	Shchelochkov et al. [2009]
34	>27.9 kb	Exons 1-3	–	Female	Shchelochkov et al. [2009]
35		Exons 1-5	–	Female	Azevedo et al. [2006]
36		Exons 1-8	–	Neonatal	Yamaguchi et al. [2006]
37		Exons 1-9	–	Neonatal	Yamaguchi et al. [2006]
38	15921 bp	Exon 2	c.78-3544_217-126del15921	Female	Quental et al. [2009]
39		Exons 2-4	–	Female	Yamaguchi et al. [2006]
40	9100 bp	Exons 2-4	c.77+6024_c.216+474del9095	Neonatal	Engel et al. [2008]
41		Exon 3	–	Neonatal	Yamaguchi et al. [2006]
42	35,832 bp	Exons 4-9	c.298+8029_c.1--5+1740del35,832	Female	Engel et al. [2008]
43		Exons 4-10	–	Neonatal	Yamaguchi et al. [2006]
44		Exons 5-6	g.42940_52876delinsT	Neonatal	Storkanova et al. [2013]
45		Exons 5-8	–	Late; somatic mosaic	Yamaguchi et al. [2006]
46	10,862 bp	Exons 6-9	c.541-600_1005 + 1880del10862	Female	Quental et al. [2009]
47		Exons 7-8	–	Neonatal	Suess et al. [1992]
48		Exon 7-9	–	Late; somatic mosaic	Yamaguchi et al. [2006]
49		Exon 9	–	Female	Yamaguchi et al. [2006]
50		Exon 9	–	Neonatal	Suess et al.[1992]
51		Exons 9-10	g.57467_82002delinsCCT	Female	Storkanova et al. [2013]
52		Partial OTC deletion	–		Rozen et al. [1985]

[a]HGVS description not available.

[b]First report of the mutation.

Table S3

Table S3. Rare polymorphisms (MAF<1%) in the OTC gene.

No.	Region	Codon[a]	Nucleotide change[a]	Amino acid or other change	Predicted effect SIFT/PolyPhen2[b]	Allele frequency	Reference/Submitter	SNP ID
1	5'-UTR		c.-100G>A	NA		–	dbSNP build 138	rs368806417
2	5'-UTR		c.-48G>T	NA		T: 0.06% (1/1659); G: 99.94% (1658/1659)	1000 Genomes	rs182454762
						T: 0.03% (3/10561); G: 99.97% (10558/10561)	NHLBI_WES	
3	Exon 1	12	c.34G>A	p.Ala12Thr	0.17/0		Tanaka et al. [2005]	
4		23	c.68G>A	p.Arg23Gln	0.24/0.139	A: 0.01% (1/10561); G: 99.99% (10560/10561)	NHLBI_WES	rs148660170
5	Exon 2	26	c.78G>A	p.Arg26Arg	Likely to disturb normal splicing	A: 0.01% (1/10561); G: 99.99% (10560/10561)	NHLBI_WES	rs141273695
6		28	c.83G>A	p.Gly28Glu	0.02/ 0.999	A: 0.01% (1/10561); G: 99.99% (10560/10561)	NHLBI_WES	rs199858968
7		43	c.127C>A	p.Leu43Ile	0.13/0.963	A: 0.01% (1/10561); C: 99.99% (10559/10560)	NHLBI_WES	rs72554309
9		49	c.147C>T	p.Thr49Thr	Unknown	T: 0.01% (1/10560); C: 99.99% (10559/10560)	NHLBI_WES	rs144153859
10		66	c.196A>C	p.Arg66Arg	Unknown	C: 0.02% (2/10559); A: 99.98% (10557/10559)	NHLBI_WES	rs369756595
11	Intron 2		c.217-54insA			–	Matsuura et al. [1993]c	
12			c.217-54A>G			G: 0.06% (1/1654); A: 99.94% (1653/1654)	1000 Genomes	rs192825987
13	Exon 3	78	c.234A>G	p.Gln78Gln	Unknown	G: 0.00%; A: 100% (35 alleles screened)	Climent & Rubio [2002]; Hata et al. [1988]	
14		91	c.272C>T	p.Thr91Ile	0.00/0.553	T: 0.01% (1/10561); C: 99.99% (10560/10561)	NHLBI_WES	rs372906667
15		96	c.286T>C	p.Ser96Pro	0.00/1	G: 0.06% (1/1659); T: 99.94% (1658/1659)	1000 Genomes	rs184053962
16	Exon 4	101	c.303A>T	p.Leu101Phe[b]	0.33/0.208	T: 1%; A: 99% (79 alleles screened)	Tuchman & Plante [1995]; Plante & Tuchman [1998][d]	
17		124	c.372C>T	p.Leu124Leu	Unknown	T: 0.06% (1/1659); C: 99.94% (1658/1659)	1000 Genomes	rs201070935
19		125	c.375G>A	p.Thr125Thr	Unknown	A: 0.01% (1/10561); G: 99.99% (10560/10561)	NHLBI_WES	rs371075231
21	Intron 4		c.386+8A>G			G: 2%; A: 98% (49 alleles screened)	Plante & Tuchman [1998][e]	rs1800325
						–	dbSNP build 138	
22	Exon 5	130	c.390A>G	p.Val130Val	Likely to disturb normal splicing	G: 0.06% (1/1659); A: 99.94% (1658/1659)	1000 Genomes	rs149795059
						G: 0.02% (2/10651); A: 99.98% (10559/10651)	NHLBI_WES	
24		151	c.453G>T	p.Leu151Leu	Unknown	T: 0%; G: 100% (51 alleles screened)	Climent & Rubio [2002]; Hata et al. [1988]	
25		177	c.529C>T	p.Leu177Phe	0.02/1.0	T: 0.12% (2/1659); C: 99.88% (1657/1659)	1000 Genomes	rs148961194
						T: 0.01% (1/10561); C: 99.99% (10560/10561)	NHLBI_WES	
27		194	c.580A>T	p.Ile194Phe	0.00/0.999	T: 0%; A: 100% (46 alleles screened)	Climent & Rubio [2002]; Hata et al. [1988]	
28		194	c.582C>T	p.Ile194Ile	Unknown	T: 0.01% (1/10561); C: 99.99% (10560/10561)	NHLBI_WES	rs200564773

29		195	c.585G>A	p.Gly195Gly	Likely to disturb normal splicing	A: 0%; G: 100% (53 alleles screened) –	Tuchman et al. [1998] dbSNP build 138	rs1800327
30		209	c.625G>T	p.Ala209Ser	0.07/0.99	T: 0.01% (1/10561); G: 99.99% (10560/10561)	NHLBI_WES	rs372179665
31		209	c.626C>G	p.Ala209Gly	0.08/ 0.999	G: 0.01% (1/10561); C: 99.99% (10560/10561)	NHLBI_WES	rs72558417
32	Exon 7	224	c.670G>C	p.Glu224Gln	0.03/ 0.994	C: 0.01% (1/10561); G: 99.99% (10560/10561)	NHLBI_WES	rs373196047
33		225	c.675G>A	p.Pro225Pro	Likely to disturb normal splicing	A: 0.01% (1/10561); G: 99.99% (10560/10561)	NHLBI_WES	rs376129619
34	Exon 8	251	c.751T>C	p.Leu251Leu	Unknown	C: 0.24% (4/1659); T: 99.76% (1655/1659) C: 0.76% (80/10561); T: 99.24% (10481/10561)	1000 Genomes NHLBI_WES	rs36005267
35		258	c.773A>G	p.Asn258Ser	0.04/0.688	G: 0.01% (1/10561); A: 99.99% (10560/10561)	NHLBI_WES	rs142592280
36		270	c.808C>G	p.Gln270Glu	0.01/0.923	G: 0.12% (2/1659); C: 00.88% (1657/1659)	1000 Genomes	rs72558451
37		279	c.837G>A	p.Gln279Gln	Likely to disturb normal splicing	A: 0%; G: 100% (69 allelels screened)	Climent & Rubio [2002]; Hata et al. [1988]	
38		285	c.855G>A	p.Gln285Gln	Likely to disturb normal splicing	A: 0%; G: 100% (69 allelels screened)	Climent & Rubio [2002]; Hata et al. [1988]	
39	Exon 9	305	c.915C>G	p.Pro305Pro	Likely to disturb normal splicing	G: 0.01% (1/10561); C: 99.99% (10560/10561)	NHLBI_WES	rs368485863
40		314	c.941A>C	p.Glu314Ala	0.12/0.003	C: 0.07% (8/10561) A: 99.93% (10553/10561)	NHLBI_WES	rs137899554
41		319	c.955C>T	p.Pro319Ser	0.09/0.024	T: 0.01% (1/10561); C: 99.99% (10560/10561)	NHLBI_WES	rs372070932
42		333	c.997A>G	p.Thr333Ala	0.09/0.982	–	Matsuura et al. [1993]; Climent & Rubio [2002]	
43	Intron 9		c.1006-12G>T			T: 0%; G: 100% (35 alleles screened)	Climent & Rubio [2002]	
44	Exon 10	338	c.1012A>C	p.Met338Leu	0.22/B0.012	C: 0.01% (1/10561); A: 99.99% (10560/10561)	NHLBI_WES	rs199568993

[a]Nucleotide +1 is the A of the translation initiation codon of the NM_000531.3.

[b]Amino acud replacements with SIFT score less than 0.05 is considered to be deleterious. Amino acid replacements with PolyPhen2 scores 0.85-1, 0.2-0.85 and 0-0.2 are considered probably damaging, possibly damaging and benign, respectively.

[c]Variant probably seen in a single individual. A different variant c.217-54A>G seen in one individual in 1000G project.

[d]Leucine seen at position 101 in Hata et al. (1988) J Biochem, 103: 302-8. Reference sequence has phenylalanine at that position.

[e]Variant seen in patients referred for mutation analysis of the OTC gene.

Table S4. Disease onset, gender and liver transplant status of OTC patients with neonatal and late onset OTCD enrolled in the longitudinal study of urea cycle disorders.

			Liver transplant		
Disease onset	**Gender**		**Yes**	**No**	**Unknown**
Neonatal	Male	32	15	17	
	Female	5	1	3	1
Late	Male	36	2	34	
	Female	92	2	90	

Table S5. Patients with OTCD who did not receive liver transplant and had symptoms of liver dysfunction at any point during longitudinal study of urea cycle defects.

			Elevated ALT activity		
Disease onset	**Gender**		**Mild[a]**	**Moderate[b]**	**Severe[c]**
Neonatal	Male	32	8	2	1
	Female	5	2	0	0
Late	Male	36	15	0	0
	Female	92	41	5	3
Asymptomatic	Male	6	1	0	0
	Female	88	18	3	0

[a]Mild elevation of ALT, defined by the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, was considered to be between 35–105 IU in female and 40–120 IU in male patients.
[b]Moderate elevation of ALT, defined by the CTCAE v4.0, was considered to be 105-175 IU in females and 120-200 IU in males.
[c]Severe elevation of ALT, defined by the CTCAE v4.0, was considered to be >175 IU in females and >200 IU in males.

Table S6. Patients with OTCD who did not receive liver transplant and had elevated plasma INR at any point during longitudinal study of urea cycle defects.

			Elevated INR		
Disease onset	**Gender**		**Mild [a]**	**Moderate[b]**	**Severe[c]**
Neonatal	Male	32	0	4	0
	Female	5	1	0	0
Late	Male	36	2	5	0
	Female	92	9	10	1
Asymptomatic	Male	6	2	0	0
	Female	88	1	7	3

[a]Mild elevation of INR, defined by the CTCAE v4.0, was considered to be between 1.2 and 1.5.
[b]Moderate elevation of INR, defined by the CTCAE v4.0, was considered to be 1.5-2.5.
[c]Severe elevation of INR, defined by the CTCAE v4.0, was considered to be >2.5.